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(54) Title: DIAGNOSTIC ANTI-PD-L1 ANTIBODY AND USE THEREOF

(57) Abstract: The present invention provides for an antibody or antigen-binding fragment thereof which binds to an epitope within human PD-L1 with high specificity and reproducibility. The inventive antibody or antigen-binding fragment thereof may be used in assessing PD-L1 expression in tissue samples to aid in patient stratification. The present invention further provides methods of producing the inventive antibody.



5 Diagnostic anti-PD-L1 antibody and use thereof

### FIELD OF THE INVENTION

The present invention pertains to the field of cancer diagnostics, in particular to the use of a diagnostic antibody to detect the presence of a PD-L1 epitope in a tumor sample for in vitro diagnosis.

### 10 BACKGROUND

Programmed cell death 1 ligand 1 (PD-L1) also known as cluster of differentiation (CD274) or B7 homolog 1 (B7-H1) is a protein that in humans is encoded by the CD274 gene and is, next to PD-L2 (CD273), one of the ligands of PD-1 (CD279). PD-L1 is a 40kDa type 1 transmembrane protein which is expressed on many hematopoietic cells, including dendritic  
15 cells, macrophages, mesenchymal stem cells and bone marrow-derived mast cells. PD-L1 is found to be inducibly expressed on epithelial and endothelial cells through the action of interferons. Sites of immune privilege such as syncytiotrophoblasts in the placenta and in the retina were found to constitutively express PD-L1.

Expression of PD-L1 in the placenta increases at the beginning of the second trimester, is  
20 upregulated by increased oxygen and is rapidly lost with low oxygen concentrations. Experiments have shown that the PD-1–PD-L pathway regulates the balance between the stimulatory and inhibitory signals needed for effective immune responses to microbes and maintenance of self-tolerance, respectively. Many microorganisms that cause chronic infection exploit the PD-1—PD-L1 pathway to evade host immune effector mechanisms. Based on a  
25 mouse model of liver infection, the PD-PD-L1 pathway is also thought to regulate immune-mediated tissue damage during viral infection, since *PD-1* null mice show increased liver damage upon clearance of adenovirus compared to wild type mice, which is thought to be caused by highly active and aggressive T-cells.

Immune attack via IFN $\gamma$  release leads to inducible upregulation of PD-L1 by mucosa creating  
30 an “immune shield” to protect against autoimmune attack in the setting of chronic inflammation or infection. Upregulated PD-L1 on these cells binds to PD-1 on T-cells contributing to the development of T-cell exhaustion.

Tumor cells have co-opted this PD-1 - PD-L1 regulatory mechanism, which under normal physiological setting protects mucosa from autoimmune attack, and instead overexpress PD-L1 to avoid immunologic surveillance thereby promoting cancer growth.

This immunosuppressive mechanism can be hijacked by PD-L1 positive tumor cells eventually leading to the escape of tumors from the elimination by the immune system. Inhibiting the PD-1/PD-L1 interaction by means of a monoclonal antibody provides a promising concept for the treatment of tumors with PD-L1 expression. Clinical trials of blocking monoclonal antibodies against PD-1 and PD-L1 are currently ongoing for patients suffering from various malignancies.

Statistical analysis of published anti-PD-L1 clinical trial data comparing the clinical response rate of PD-L1 positive or PD-L1 negative patients indicates that PD-L1 expression is a predictive marker of clinical response for certain cancer types and a correlation biomarker for others (Gandini et al., Crit Rev Oncol Hematol. 2016 Apr;100:88-98). In metastatic melanoma, for example, PD-L1 expression in tumor tissue is associated with a significant better prognosis, as PD-L1 positive patients receiving anti-PD-L1 therapy show a 53% reduction in mortality.

Studies have also shown that across multiple cancer types responses to anti-PD-L1 therapy were observed in patients with tumors expressing high levels of PD-L1, in particular when PD-L1 was expressed on tumor-infiltrating immune cells (see e.g. Herbst et al., Nature. 2014 Nov 27;515(7528):563-7; Ilie et al., Annals of Oncology 27: 147–153, 2016). In papillary thyroid cancer PD-L1 expression was found to correlate with recurrence and shortened disease free survival supporting the use of PD-L1 expression in this tumor type as a prognostic marker (Chowdhury et al., Oncotarget. 2016 Apr 12).

Detection and scoring of PD-L1 expression in tumor tissue samples is usually done by means of immunohistochemistry on frozen or formalin-fixed, paraffin-embedded (FFPE) tumor tissue sections. Scoring of PD-L1 expression can done using different methodologies: One approach for example employed a binary end-point scoring of a specimen of being positive or negative for PD-L1 expression, with a positive result defined in terms of the percentage of tumor cells that exhibited histologic evidence of cell-surface membrane staining. Two different cut-off values of 1% and 5% of total tumor cells have been used at which a tumor specimen was scored as PD-L1 positive (Cancer. 2011 May 15;117(10):2192-201; N Engl J Med 2012;366:2443-54.). PD-L1 expression in tumor specimens has also been quantified by scoring both, tumor cells and tumor-infiltrating immune cells that display a membranous staining, compared to tumor cells which showed both, membranous and a prominent cytoplasmic staining. Specimen scoring based on PD-L1 expression was subsequently done, whereby IHC scores of 0, 1, 2, or 3 were given. A specimen was scored with "0" if less than 1% of the cells were PD-L1 positive, "1" for more than 1%, but less than 5%, "2" if more than

5%, but less than 10%, or “3” if more than 10% of the cells were PD-L1 positive (Herbst et al. Nature. 2014 Nov 27;515(7528):563-7). PD-L1 expression in tumor-infiltrating mononuclear cells (TIMCs) has been assessed using a semi-quantitative approach according to three categories with respective scores of 0, 1, or 2 depending on the number of TIMCs in the specimen: 0% = 0, <5% = 1, ≥5% = 2.

The specificity and reproducibility of most commercially available anti-PD-L1 antibodies has not been thoroughly assessed and limitations of some widely used antibodies have been reported (see e.g. Cancer 2011;117: 2192–201; Carvajal-Hausorf et al., Laboratory Investigation (2015) 95, 385–396). For example, WO 2014/165422 A1, WO 2016/007235 A1 disclose an anti-PD-L1 antibodies and scoring guidelines for use with the respective antibody to assess PD-L1 expression.

There is thus a continued need to expand the repertoire in available anti-PD-L1 antibodies that are highly specific for PD-L1 and that yield reproducible results to aid in the stratification of tumor patients amenable for an anti-PD-L1-based therapy.

## SUMMARY OF THE INVENTION

The present inventors have surprisingly found that an antibody or antigen-binding fragments thereof directed against an epitope comprised in the amino acid sequence according to SEQ ID NO:1 binds to human PD-L1 with high specificity and reproducibility.

In a first embodiment the present invention provides an antibody or antigen binding fragments thereof that binds to an epitope comprised in the amino acid sequence according to SEQ ID NO: 1 with high specificity and yields reproducible results, whereby the antibody or antigen-binding fragment thereof comprises at least three amino acid sequences of SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 in its light chain sequence at least three amino acid sequences of SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 in their heavy chain sequence.

The inventive antibody or antigen-binding fragment thereof may according to one embodiment be a Fab fragment.

According to one embodiment the inventive antigen-binding fragment is a F(ab')<sub>2</sub> fragment.

According to one embodiment the inventive antigen-binding fragment is a Fab' fragment.

In one embodiment the inventive antibody is a scFv.

According to one embodiment the inventive antibody is a di-scFv.

According to one embodiment the inventive antibody is a monoclonal antibody.

According to one embodiment the inventive antibody is an IgG type antibody.

According to one embodiment the inventive antibody light chain or antigen binding fragment thereof comprises all of SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8.

According to one embodiment the heavy chain of the inventive antibody or antigen binding fragment thereof comprises all of SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, .SEQ ID NO: 13, SEQ ID NO: 14.

In one embodiment the inventive antibody comprises a heavy chain or antigen-binding fragment thereof comprising the amino acid sequence according to SEQ ID NO: 110 and a light chain or antigen-binding fragment thereof comprising the amino acid sequence according to SEQ ID NO: 111.

According to a preferred embodiment the light chain of the inventive antibody comprises the amino acid sequence according to SEQ ID NO: 15 and the heavy chain comprises the amino acid sequence according to SEQ ID NO: 16.

In a preferred embodiment the inventive antibody light chain comprises the amino acid sequence according to SEQ ID NO: 114 and the antibody heavy chain comprises the amino acid sequence according to SEQ ID NO: 115.

In a preferred embodiment the inventive antibody is a rabbit antibody, or a rabbit-derived antibody.

According to one embodiment the inventive antibody or antigen-binding fragment thereof as disclosed above is further coupled to a detectable label.

According to one embodiment the detectable label of the inventive antibody or antigen-binding fragment thereof is one of an enzyme, or fluorophore, or enzyme substrate.

According to one embodiment the inventive antibody or antigen-binding fragment thereof as disclosed above is for use in detecting the presence or expression of an epitope comprised in SEQ ID NO: 1 in a sample, which according to one embodiment is a biological sample, preferably, a tissue sample, fixed tissue sample, or a formaldehyde-fixed paraffin-embedded (FFPE) tissue, more preferably, a tumor-derived formaldehyde-fixed paraffin-embedded (FFPE) tissue.

In one embodiment the detection of the presence or absence of an epitope comprised in SEQ ID NO: 1 as disclosed above is done by means of flow cytometry, ELISA, or Western blotting using the inventive antibody or antigen-binding fragment thereof as disclosed above.

According to a preferred embodiment the detection of the presence or expression of an epitope comprised in SEQ ID NO: 1 using the inventive antibody or antigen-binding fragment thereof as disclosed above is done by means of immunohistochemistry (IHC).

5 In one embodiment the present invention provides for a method of detecting the presence or expression of human PD-L1 or any fragment thereof in a sample which comprises the amino acid sequence according to SEQ ID NO: 1 whereby the inventive method comprises the step of contacting the sample with the inventive antibody or antigen-binding fragment thereof and detecting the presence of bound antibody or antigen binding fragment thereof.

10 In one embodiment the inventive in vitro method as disclosed above is used on a biological sample, tissue sample, a fixed tissue sample, preferably a formaldehyde-fixed paraffin-embedded (FFPE) tissue sample, more preferably a formaldehyde-fixed paraffin-embedded (FFPE) tumor tissue sample.

15 According to one embodiment the sample used in the inventive in vitro method as disclosed above is derived from a subject having, or at risk of cancer, T-cell dysfunction, acute or chronic infection or tumor immunity.

In one embodiment the present invention pertains to the use of the inventive antibody or antigen-binding fragment as disclosed above in the presence or expression of an epitope comprised in SEQ ID NO: 1 in a sample.

20 In one embodiment the present invention provides an isolated polynucleotide encoding the inventive antibody or antigen binding fragment thereof as disclosed above.

According to one embodiment the present invention provides an expression vector which comprises the the polynucleotide encoding the inventive antibody or antigen-binding fragment thereof.

25 In one embodiment the present invention provides for an expression vector as disclosed above for use in producing the inventive antibody.

In one embodiment the present invention provides at least one host cell comprising at least one expression vector according to the invention.

In one embodiment the present invention provides at least one host cell according to the invention for use in the manufacture the inventive antibody.

30 According to one embodiment the present invention provides a method of treating cancer in patient which comprises the steps of detecting the presence or expression of human PD-L1 in a sample from said patient using the inventive anti-PD-L1 antibody as disclosed above, comparing the PD-L1 expression in the patient sample to a reference sample and

administration of an immune checkpoint inhibitor (e.g. an anti-PD-L1 or anti-PD-1 antibody) to the patient if the PD-L1 expression in the patient sample is increased compared to the reference sample.

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**BRIEF DESCRIPTION OF DRAWINGS**

**Figure 1:** ELISA results for inventive antibody clone MKP1A07310 using 50µl/well of immunogen at a concentration of 1µg/ml for coating of the wells of the ELISA plate.

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**Figure 2:** Western Blot using the inventive antibody clone MKP1A07310 at dilutions of 1:10000 and 1:25000 and a control antibody (anti-CD247, dilution 1:250) using the cell lysates indicated. Western blotting was done using cell lysates of PD-L1 transfected HEK293, PD-L1 expressing MDA-MB 231, siRNA PDL1 knockdowns of MDA-MB 231 and the PD-L1 low cell line A549.

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**Figure 3:** Immunohistochemical staining using the inventive antibody (MKP1A07310) and a different anti-PD-L1 antibody directed against an extracellular epitope of human PD-L1 in a siRNA knockdown on MDA-MB 231 cells. PD-L1 staining was reduced in the siRNA knockdown cells.

20

**Figure 4:** The inventive antibody (MKP1A07310) as well as one antibody directed against an extracellular epitope of human PD-L1 (MKP1B19610) were characterized using MKP1A7310 in a concentration of 1µg/ml, and MKP1B19610 at three different concentrations (2µg/ml, 5 µg/ml and 10 µg/ml) on a cancer cell line array out of 70 cell lines to assess and compare their respective binding characteristics and to determine the optimum working concentration for further studies. The cancer cell line array (CAX09\_70\_MB, manufactured by Multiblock GmbH and Zytomed Systems GmbH) was used for the assessment of antibody properties in IHC. The cancer cell lines that were used for the manufacture of the custom cancer cell line array were fixed in phosphate buffered 4% paraformaldehyde, pH 7 over 16 to 48 hours at room temperature and subsequently embedded in paraffin.

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**Figure 5:** Intra-run variability of the inventive antibody (MKP1A07310) on tissue array CAX08\_60, correlation coefficients of  $r = 0.99$  between different slides.

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**Figure 6:** Characterization of the inventive antibody (MKP1A07310, marked by "#") and one antibody directed against an extracellular epitope of human PD-L1 (MKP1B19610) on the cancer cell line array CAX09\_70 were tested with

MKP1A7310 at a concentration of 1µg/ml and MKP1B19610 at a concentration of 10 µg/ml.

**Figure 7:** (A) IHC detection of PD-L1 positive cells using the inventive antibody with DAB as chromogen (right image); detection of chromogen by computer software (middle image); detection of blue staining of nuclei and cytoplasm (right image). Arrows indicate positive cells. (B) IHC staining on positive and negative control tissues using the inventive antibody MKP1A07310 at a concentration of 1µg/ml.

**Figure 8:** (A) Immunohistochemical using the inventive antibody MKP1A07310 on FFPE tissue of the xenograft tissue microarray TMA\_Xenos\_12 at a concentration of 2µg/ml. (B) Immuno-histochemical staining using an antibody directed against an extracellular epitope of human PD-L1 (MKP1B19610) at a concentration of 10µg/ml on FFPE tissue of the xenograft tissue microarray TMA\_Xenos\_12. Exemplary PD-L1 positive cells are indicated by arrows

**Figure 9:** (A) Immunohistochemical staining using the inventive antibody MKP1A07310 at a concentration of 1µg/ml on FFPE tissue of the cancer cell line array CAX08\_60. (B) Immunohistochemical staining using an antibody directed against an extracellular epitope of human PD-L1 (MKP1B19610) at a concentration of 10µg/ml on FFPE tissue of the cancer cell line array CAX08\_60. Arrows indicate exemplary PD-L1 positive cells.

**Figure 10:** Immunohistochemical staining using the inventive antibody MKP1A07310 at a concentration of 1µg/ml, and the antibody MKP1B19610 directed against an extracellular epitope of PD-L1 at a concentration of 10 µg/ml on a discovery XT staining instrument on FFPE fixed cell lines NCI-H2009, Lox and A2780 (NCI-H2900, Lox cancer cell lines express high levels of PD-L1, cancer cell line A2780 only minor amounts of PD-L1).

**Figure 11:** Immunohistochemical staining using the inventive antibody MKP1A07310 at a concentration of 0.5 µg/ml, and the antibody MKP1B19610 directed against an extracellular epitope of PD-L1 at a concentration of 1 µg/ml on an Autostainer Link staining instrument on FFPE fixed cell lines NCI-H2009, Lox and A2780.

**Figure 12:** Correlation of IHC results using MKP1B19610 on FFPE cancer cell lines stained using the Discovery XT® System (Ventana) and the AutostainerLink system (DAKO). (A) results of the IHC runs using MKP1B19610 at low and high pH



using the different IHC platforms and concentrations as indicated plotted against each other. (B) Statistical analysis of the diagrams, with Pearson correlation coefficients of 0.6186 (2µg/ml, high pH), 0.6969 (1µg/ml, high pH) and at low pH 0.4309 (5µg/ml), 0.6631 (2µg/ml).

5 **Figure 13:** (A) Correlation of IHC results using the inventive anti-PD-L1 antibody MKP1A07310 on FFPE cancer cell lines stained on a Discovery XT ® System (Ventana) or AutostainerLink (DAKO) at high pH using the antibody concentrations as indicated. (B) Statistical analysis of the correlation of the IHC results with correlations coefficients of  $r=0.9474$  (at 1µg/ml) and  $r=0.9695$  (at 10 2µg/ml).

**Figure 14:** (A) IHC correlation between the anti-PD-L1 antibody MKP1B19610 and the inventive antibody MKP1A07310 on FFPE cancer cell lines stained with the AutostainerLink (DAKO) at the concentrations indicated (diamonds: 1µg/ml; circles: 2µg/ml), the inventive antibody was used at a concentration of 0.5µg/ml as indicated. (B) statistical analysis of the correlation of the IHC results with 15 correlations coefficients of  $r=0.6503$  (at 2µg/ml MKP1B19610) and  $r=0.7088$  (at 1µg/ml MKP1B19610).

20

### SEQUENCE LISTING

SEQ ID NO: 1	RLRKGRMMDVKKCGIQDTNSKKQSDTHLEET
SEQ ID NO: 2	CGIQDTNSKKQSDTH
SEQ ID NO: 3	AQVLTQTPSPVSASVGSTVTINCQAS
SEQ ID NO: 4	QSLHRNNY
SEQ ID NO: 5	LSWFQQKPGQPPKQLIY
SEQ ID NO: 6	QAS
SEQ ID NO: 7	TLASGVSSRFSGSGSGTQFTLTISDVVCDDAATYYC
SEQ ID NO: 8	LGGVSGGPYP
SEQ ID NO: 9	EQLVESGGGLVTPGGSLLTCTVS
SEQ ID NO: 10	TIDLSTFA
SEQ ID NO: 11	ISWVRQAPGKGLEWIGT
SEQ ID NO: 12	INTDLTT
SEQ ID NO: 13	YYVNWAKGRFTISKTSSTTVDLKMTGLTIEDTATYFC

SEQ ID NO: 14	ARKLFGNGNV
SEQ ID NO: 15	MDTRAPTQLLGLLLLWLPGATVAQVLTQTPSPVSASVGSTVTINC QASQSLHRNNYLSWFQQKPGQPPKQLIYQASTLASGVSSRFSGSG SGTQFTLTISDVVCDDAATYYCLGGVSGGPYPFGGGTEVVVKGDP VAPTVLIFPPAADQVATGTVTIVCVANKYFPDVTVTWEVDGTTQT TGIENSKTPQNSADCTYNLSSTLTSTQYNHKEYTCKVTQGT SVVQSFNRGDC
SEQ ID NO: 16	METGLRWLLLVAVLKGVQCQEQLVESGGGLVTPGGSLTLTCTVST IDLSTFAISWVRQAPGKGLEWIGTINTDLTTYVNWAKGRFTISK TSSTTVDLKMTGLTIEDTATYFCARKLFGNGNVWGPGLVTVSSG QPKAPSVFPLAPCCGDTSPSTVTLGCLVKGYLPEPVTVTWNSGTL TNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPVTCNVAHPATNTKV DKTVAPSTCSKPTCPPPELLGGPSVFI FPPKPKDTLMISRTPEVT CVVVDVSQDDPEVQFTWYINNEQVRTARPLREQQFNSTIRVVST LPIAHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLEPKVYT MGPPREELSSRSVSLTCMINGFYPSDISVEWEKNGKAEDNYKTP AVLDSGSGSYFLYSKLSVPTSEWQRGDVFTCSVMHEALHNHYTQKS ISRSPGK
SEQ ID NO: 17	5'-atggacacga gggccccac tcagctgctg gggctcctgc tgctctggct cccaggtgcc acagttgccc aagtgtgac ccagacccca tccccgtgt ctgcatctgt gggaagcaca gtcaccatca attgccaggc cagtcagagt cttcatcgca acaactactt atcctggttt cagcagaaac cagggcagcc tccaagcaa ctgatctatc aggcattccac tctggcatct ggggtctcat cgcggttcag tggcagtgga tctgggacac agttcactct caccatcagc gatgtggtgt gtgacgatgc tgccacttac tactgtctgg gcggtgttag tggtggctct tatcctttcg gcggaggagc cgaggtgggtc gtcaaagggtg atccagttgc acctactgtc ctcatcttcc caccagctgc tgatcagggtg gcaactggaa cagtcacat cgtgtgtgtg gcgaataaat actttcccga tgtcaccgtc acctgggagg tggatggcac cacccaaaca actggcatcg agaacagtaa aacaccgcag aattctgcag attgtaccta caacctcagc agcactctga cactgaccag cacacagtac aacagccaca aagagtacac ctgcaagggtg acccagggca cgacctcagt cgtccagagc ttcaataggg gtgactgtta g
SEQ ID NO: 18	5'-atggagactg ggctgcgctg gcttctcctg gtcgctgtgc tcaaagggtg ccagtgctcag gagcagctgg tggaatccgg aggaggcctg gtcacgcctg ggggatccct gacactcacc tgcacagtct ctacaatcga cctcagtacc tttgcaataa gctgggtccg ccaggctcca ggggaaggggc tggagtggat cggaaccatt aatactgata ttaccacata ctatgtgaat tgggcgaaag gccgattcac catctccaaa

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SEQ ID NO: 21	RKGRMMDV
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SEQ ID NO: 23	GRMMDVKK
SEQ ID NO: 24	RMMDVKKC
SEQ ID NO: 25	MMDVKKCG
SEQ ID NO: 26	MDVKKCGI
SEQ ID NO: 27	DVKKCGIQ
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SEQ ID NO: 32	GIQDTNSK
SEQ ID NO: 33	IQDTNSKK

SEQ ID NO: 34	QDTNSKKQ
SEQ ID NO: 35	DTNSKKQS
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SEQ ID NO: 37	NSKKQSDT
SEQ ID NO: 38	SKKQSDTH
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SEQ ID NO: 92	GRMMDVKKCGI
SEQ ID NO: 93	RMMDVKKCGIQ
SEQ ID NO: 94	MMDVKKCGIQD
SEQ ID NO: 95	MDVKKCGIQDT
SEQ ID NO: 96	DVKKCGIQDTN
SEQ ID NO: 97	VKKCGIQDTNS
SEQ ID NO: 98	KKCGIQDTNSK
SEQ ID NO: 99	KCGIQDTNSKK
SEQ ID NO: 100	CGIQDTNSKKQ
SEQ ID NO: 101	GIQDTNSKKQS

SEQ ID NO: 102	IQDTNSKKQSD
SEQ ID NO: 103	QDTNSKKQSDT
SEQ ID NO: 104	DTNSKKQSDTH
SEQ ID NO: 105	TNSKKQSDTHL
SEQ ID NO: 106	NSKKQSDTHLE
SEQ ID NO: 107	SKKQSDTHLEE
SEQ ID NO: 108	KKQSDTHLEET
SEQ ID NO: 109	5'-augauaauauggccacaaccaug
SEQ ID NO: 110	EQLVESGGGLVTPGGSLTLCTVSTIDLSTFAISWVRQAPGKGLE WIGTINTDLTTYVNWAKGRFTISKTSSTTVDLKMTGLTIEDTAT YFCARKLFGNGNVWGPGLTVTVSS
SEQ ID NO: 111	AQVLTQTPSPVSVASVGSTVTINCQASQSLHRNNYLSWFQQKPGQP PKQLIYQASTLASGVSSRFSGSGSGTQFTLTISDVVCDDAATYYC LGGVSGGPYPFGGGTEVVVK
SEQ ID NO: 112	MDTRAPTQLLGLLLLWLPATVA
SEQ ID NO: 113	METGLRWLLLVAVLKGVQC
SEQ ID NO: 114	QVLTQTPSPVSVASVGSTVTINCQASQSLHRNNYLSWFQQKPGQPP KQLIYQASTLASGVSSRFSGSGSGTQFTLTISDVVCDDAATYYCL GGVSGGPYPFGGGTEVVVKGDPVAPTIVLIFPPAADQVATGTVTIV CVANKYFPDVTVTWEVDGTTQTGTIENSKTPQNSADCTYNLSSTL TLTSTQYNHKEYTCKVTQGTTSVVQSFNRGDC
SEQ ID NO: 115	QEQLVESGGGLVTPGGSLTLCTVSTIDLSTFAISWVRQAPGKGL EWIGTINTDLTTYVNWAKGRFTISKTSSTTVDLKMTGLTIEDTA TYFCARKLFGNGNVWGPGLTVTVSSGQPKAPSVFPLAPCCGDTPS STVTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVRQSSGLYSL SSVSVTSSSQPVTTCNVAHPATNTKVDKTVAPSTCSKPTCPPPEL LGGPSVFI FPPKPKDTLMISRTPEVTCVVVDVSQDDPEVQFTWYI NNEQVRTARPPLREQQFNSTIRVVSTLPIAHQDWLRGKEFKCKVH NKALPAPIEKTISKARGQPLEPKVYTMGPPREELSSRSVSLTCMI NGFYPSDISVEWEKNGKAEDNYKTTPAVLDSGYSFLYSKLSVPT SEWQRGDVFTCSVMHEALHNHYTQKSISRSPGK
SEQ ID NO: 116	FGGGTEVVVK
SEQ ID NO: 117	WGPGLTVTVSS

### DETAILED DESCRIPTION OF THE INVENTION

Although the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodologies, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined

otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

In the following, the elements of the present invention will be described. These elements are listed with specific embodiments, however, it should be understood that they may be combined  
5 in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations and  
10 combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise.

Throughout this specification and the claims which follow, unless the context requires otherwise, the term "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated member, integer or step but not the exclusion of  
15 any other non-stated member, integer or step. The term "consist of" is a particular embodiment of the term "comprise", wherein any other non-stated member, integer or step is excluded. In the context of the present invention, the term "comprise" encompasses the term "consist of".

The terms "a" and "an" and "the" and similar reference used in the context of describing the invention (especially in the context of the claims) are to be construed to cover both the singular  
20 and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. No language in the specification should be construed as indicating any non-claimed  
25 element essential to the practice of the invention.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether supra or infra, are hereby incorporated by reference  
30 in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

The described objectives are solved by the present invention, preferably by the subject matter of the appended claims.

The inventors have surprisingly found that the inventive antibody or antigen-binding fragment thereof is highly specific for PD-L1 and yields reproducible results in PD-L1 detection by

immunohistochemistry to aid in the stratification of tumor patients amenable for an anti-PD-L1 based therapy.

The described objectives are solved according to a first embodiment by the inventive antibody or antigen-binding fragment thereof that binds to an epitope comprised in the amino acid sequence according to SEQ ID NO:1, wherein the antibody light chain or antigen binding fragment thereof comprises at least one of the amino acid sequences according to SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 and the antibody heavy chain or antigen binding fragment thereof comprise at least one of the amino acid sequences according to SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14. For example, the antibody or antigen-binding fragment thereof according to the invention comprises in its light chain at least one of SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or e.g. two, three, four, five, or all of SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8. For example, the light chain may comprise SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or e.g. SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5; SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6; SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or e.g. SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6; SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7; SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or e.g. SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 116, and the antibody heavy chain or antigen binding fragment thereof comprise at least one of SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, e.g. two, three, four, five, or all of SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, e.g. SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 11, SEQ ID NO: 12; SEQ ID NO: 12, SEQ ID NO: 13; SEQ ID NO: 13, SEQ ID NO: 14, or e.g. SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11; SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12; SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13; SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, or e.g. SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12; SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13; SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14; or e.g. SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, or e.g. SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 117. The light chain and heavy chain amino acid sequence elements as disclosed above may e.g. be present in numerically increasing order of the SEQ ID numbers listed, and, if all of the heavy and light chain sequence elements are present in the inventive antibody or antigen-binding fragment thereof, it is preferred that the light chain sequence elements are comprised in the order SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID



NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 116 and the heavy chain sequence elements are comprised in the order of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 117. As used for the inventive antibody the term "antibody" refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as immunoglobulin variable region genes. Antibody light chains are classified as either kappa or lambda. Antibody heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. Typically, the antigen-binding region of an antibody will be most critical in specificity and affinity of binding. An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD) (see e.g. J Allergy Clin Immunol. 2010 February ; 125(2 0 2): S41-S52). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain ( $V_L$ ) and variable heavy chain ( $V_H$ ) refer to these light and heavy chains respectively.

According to one embodiment the inventive antibody or antigen-binding fragment thereof is a monoclonal antibody, an Fab,  $F(ab')_2$ , Fab', scFv, or di-scFv. Antibodies exist as intact immunoglobulins or e.g. as a well-characterized antigen-binding fragments produced by digestion with peptidases such as pepsin, or papain: Pepsin will result in proteolytic cleavage below the disulfide linkages and result in a  $F(ab')_2$  antibody fragments, while proteolytic cleavage by papain, which cleaves above the disulfide linkages, will result in two Fab fragments. Accordingly a  $F(ab')_2$  fragment is a dimer of Fab which itself is a light chain joined to  $V_H$ - $CH_1$  by a disulfide bond. The  $F(ab')_2$  may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the  $F(ab')_2$  dimer into an Fab' monomer. The aforementioned antibody fragments are defined in terms of the digestion of an intact antibody with pepsin and papain, however, such fragments may be synthesized de novo either chemically or by using recombinant DNA methodology.

The term "antigen-binding fragment thereof" according to the invention refers to (i) a Fab fragment, a monovalent fragment consisting of the  $V_L$ ,  $V_H$ ,  $CL$  and  $CH_1$  domains, (ii) a  $F(ab')_2$  fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, (iii) a Fd fragment consisting of the  $V_H$  and  $CH_1$  domains, (iv) a Fv fragment consisting of the  $V_L$  and  $V_H$  domains of a single arm of an antibody, (v) a dAb fragment (see e.g. Ward et al (1989) Nature 341 544-46), which comprises a  $V_H$  domain, and (vi) an isolated complementarity determining region (CDR). The term "scFv" as used in the present invention refers to a molecule comprising an antibody heavy chain variable domain (or region;  $V_H$ ) and

an antibody light chain variable domain (or region; VL) connected by a linker, and lacks constant domains, e.g. an scFv fragment according to the invention may e.g. include binding molecules which consist of one light chain variable domain (VL) or portion thereof, and one heavy chain variable domain (VH) or portion thereof, wherein each variable domain (or portion thereof) is derived from the same or different antibodies. scFv molecules preferably comprise an linker interposed between the VH domain and the VL domain, which may e.g. include a peptide sequence comprised of the amino acids glycine and serine. For example, the peptide sequence may comprise the amino acid sequence (Gly<sub>4</sub> Ser)<sub>n</sub>, whereby n is an integer from 1-6, e.g. n may be 1, 2, 3, 4, 5, or 6, preferably n=4. scFv molecules and methods of obtaining them are known in the art and are described, e.g., in U.S. Pat. No. 5,892,019, Ho et al. 1989. Gene 77:51; Bird et al. 1988 Science 242:423; Pantoliano et al. 1991. Biochemistry 30:10117; Milenic et al. 1991. Cancer Research 51:6363; Takkinen et al. 1991. Protein Engineering 4:837. The term "di-scFv" as used for the inventive antigen-binding fragments refer to two scFv fragments which are coupled to each other via a linker, e.g. such as disclosed in Cancer Research 54, 6176-618, December 1, 1994, or Chem Commun (Camb). 2007 Feb 21;(7):695-7.

According to one embodiment the antibody or antigen binding fragment according to the invention is a monoclonal antibody. The term "monoclonal antibody" as used for the inventive antibody, refers to an antibody obtained from a population of substantially homogeneous antibodies. Monoclonal antibodies are identical except for possible naturally occurring mutations that may be present in minor amounts, or minor differences in their glycosylation pattern. Monoclonal antibodies, such as e.g. the inventive monoclonal antibody, are highly specific, being directed against a single antigenic site and specifically bind to a single epitope within the antigen, unlike polyclonal antibody preparations which typically include different antibodies directed against different epitopes. Monoclonal antibodies may e.g. be obtained by hybridoma culture as e.g. described by Kohler et al., (1975) Nature, 256:495, or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567).

According to one embodiment the inventive antibody is an IgG type monoclonal antibody, e.g. the inventive antibody may be a human IgG1, IgG2, IgG3, or IgG4 type monoclonal antibody, or e.g. murine IgG1, IgG2a, IgG2b, IgG2c, IgG3, or e.g. rat IgG1, IgG2a, IgG2b, IgG2c. For example, the inventive IgG type monoclonal antibody may be of any origin, e.g. of murine, goat, sheep, hamster, rat, or rabbit origin.

According to a preferred embodiment the inventive antibody or the antigen-binding fragment thereof comprises all of the light chain amino acid sequences according to SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8.

In a preferred embodiment, the inventive antibody or the antigen-binding fragment thereof comprises all of the heavy chain amino acid sequences according to SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14.

5 In one embodiment, the inventive antibody or antigen-binding fragment thereof comprises three CDRs from the variable region of the light chain which comprises the amino acid sequence according to SEQ ID NO: 111 and three CDRs from variable region of the heavy chain which comprises the amino acid sequence according to SEQ ID NO:110.

10 In one preferred embodiment, the inventive antibody or antigen-binding fragment thereof comprises three CDRs from the variable region of the light chain which comprises the amino acid sequence according to SEQ ID NO: 111, and three CDRs from variable region of the heavy chain which comprises the amino acid sequence according to SEQ ID NO:110, and the CDRs of the light chain comprise or are comprised in the amino acid sequences according to SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8 and the CDRs of the heavy chain comprise or are comprised in the amino acid sequences according to SEQ ID NO: 10, SEQ ID NO: 12  
15 and SEQ ID NO: 14.

In one preferred embodiment, the inventive antibody or antigen-binding fragment thereof as disclosed above comprises a light and heavy chain which comprise the CDRs as defined above whereby the light chain variable region according to SEQ ID NO: 111 further comprises four framework regions (FR) and the heavy chain variable region according to SEQ ID NO:110  
20 further comprises four framework regions, whereby the framework regions of the light chain variable region comprise or are comprised in the amino acid sequences according to SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 116, and the framework regions of the heavy chain variable region comprise or are comprised in the amino acid sequences according to SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 117.

25 According to a preferred embodiment the heavy chain of the inventive antibody (or e.g. an antigen-binding fragment thereof) comprises a variable region which comprises the amino acid sequence according to SEQ ID NO: 110 and the light chain of the inventive antibody (or e.g. an antigen-binding fragment thereof) comprises a variable region which comprises the amino acid sequence according to SEQ ID NO: 111.

30 According to a more preferred embodiment the inventive antibody or antigen-binding fragment thereof comprises a light chain which comprises the amino acid sequence according to SEQ ID NO: 114 in which the signal sequence has been removed, e.g. the amino acid sequence according to SEQ ID NO: 112, e.g. by proteolytic cleavage and a heavy chain which comprises the amino acid sequence according to SEQ ID NO: 115 in which the signal sequence has been

removed, e.g. the amino acid sequence according to SEQ ID NO: 113, e.g. by proteolytic cleavage.

In one embodiment, the variable light chain ( $V_L$ ) and the variable heavy chain ( $V_H$ ) of the inventive antibody, e.g. comprising the amino acid sequences according to SEQ ID NO: 110 and SEQ ID NO: 111 may be comprised in a scFv, or e.g. a Fab which e.g. may be further be linked to a GA-SEED, or an AG-SEED. The term "SEED" as refers to strand-exchange engineered domain (SEED)  $C_H3$  heterodimers as disclosed in WO2007/110205 A2, Protein Engineering, Design & Selection vol. 23 no. 4 pp. 195–202, 2010. These heterodimeric molecules are derivatives of human IgG and IgA  $CH3$  domains and create complementary human SEED  $C_H3$  heterodimers that are composed of alternating segments of human IgA and IgG  $C_H3$  sequences. The resulting pair of SEED  $C_H3$  domains preferentially associates to form heterodimers in a 1:1 ratio when expressed in mammalian cells to form "SEEDbodies" (Sb). The term "GA-SEED" hereby indicates that the SEED molecule begins with an IgG sequence, followed by an IgA sequence, while "AG-SEED" refers to the fact that the SEED molecule begins with an IgA-derived sequence followed by an IgG-derived sequence. The inventive scFv comprising the amino acid sequences according to SEQ ID NO: 110 and SEQ ID NO: 110 may e.g. be linked to the GA- or AG-SEED via a peptide linker e.g. via a glycine-serine linker of the amino acid sequence  $(Gly_4 Ser)_n$  as disclosed above.

According to a more preferred embodiment the inventive antibody light chain or antigen-binding fragment thereof comprises the amino acid sequence according to SEQ ID NO: 15 and the inventive antibody heavy chain or antigen-binding fragment thereof comprises the amino acid sequence according to SEQ ID NO: 16. For example, the inventive antibody or antigen-binding fragment thereof may comprise the amino acid sequences according to SEQ ID NO: 15 in its light chain and the amino acid sequence according to SEQ ID NO: 16 in its heavy chain. For example, both heavy and light chain sequences of the inventive antibody or antigen binding fragment thereof may comprise amino acid substitutions in the amino acid sequences not comprised in any of SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 111 for the light chain and SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, .SEQ ID NO: 13, SEQ ID NO: 14, or SEQ ID NO: 110 for the heavy chain, e.g. in amino acids that do not form part of the variable region of the light or heavy chain, or e.g. any light or heavy chain CDR or framework sequences, e.g. amino-terminally or carboxy-terminally to SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or e.g. SEQ ID NO: 111 of the light chain, or SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, .SEQ ID NO: 13, SEQ ID NO: 14, or SEQ ID NO: 110 of the heavy chain of the inventive antibody. Amino acid substitutions may e.g. be non-conservative amino acid substitutions, or conservative amino acid substitutions. The term "conservative amino acid substitutions" as used in the present invention refers to an amino

acid substitution in which the substituted amino acid residue is of similar charge as the replaced residue and is of similar or smaller size than the replaced residue. Conservative substitutions of amino acids include substitutions made e.g. amongst amino acids within the following groups:

<b>Class</b>	<b>Amino acid</b>
Aliphatic	Glycine, Alanine, Valine, Leucine, Isoleucine
Hydroxyl or Sulfur/Selenium-containing	Serine, Cysteine, Selenocysteine, Threonine, Methionine
Cyclic	Proline
Aromatic	Phenylalanine, Tyrosine, Tryptophane
Basic	Histidine, Lysine, Arginine
Acidic	Aspartate, Glutamate, Asparagine, Glutamine

According to a preferred embodiment, the inventive monoclonal IgG antibody is a rabbit antibody, or e.g. a rabbit-derived antibody. The inventive rabbit IgG monoclonal antibody may e.g. be generated according to the methods disclosed in Antibodies: A Laboratory Manual, Second Edition, Chapter 7, Edited by Edward A. Greenfield, CSH Press, ISBN 978-1-936113-81-1, or e.g. according to any suitable method in the art such as those disclosed in Proc. Natl. Acad. Sci. USA, Vol. 92, pp. 9348-9352, September 1995, or e.g. US patent 7,732,168 B2. The term "rabbit-derived" as used for the inventive antibody refers to an antibody that was produced in a heterologous expression system (e.g. CHO cells, HEK293 cells) using polynucleotide sequences obtained from a rabbit hybridoma producing the respective antibody, such as e.g. the inventive antibody. For example, a rabbit-derived antibodies of the invention includes antibodies that may be produced in CHO or HEK cells transfected with an expression vector comprising polynucleotides encoding the amino acid sequence (e.g. according to SEQ ID NO: 15 and/or SEQ ID NO:16) of the antibody produced by a rabbit hybridoma cell. For example, recombinant monoclonal antibodies according to the invention may be generated from rabbit hybridoma cells producing the inventive antibody by the method as disclosed in PLoS One. 2016 Mar 29;11(3):e0152282. For example, cDNA from a single B cells may be prepared using Superscript III reverse transcriptase (Invitrogen) primed with oligo (dT). Antibody variable-region genes may then be recovered via two rounds of PCR using either KOD DNA polymerase (EMD Millipore) or TaqPlus Precision DNA polymerase (Agilent). A primary PCR may e.g. utilize gene-specific primers at both the 5' and 3' ends of the antibody variable region. The 5' oligonucleotide set may bind e.g. at the 5' end of the leader sequence

and the 3' reverse primer set may anneal to CH1 or C $\kappa$  region respectively. In the secondary PCR, a single 5' forward oligonucleotide that anneals to a "tail" encoded at the 5' end of the primary PCR product may be used with a 3' primer set that anneals in the J region. The secondary oligonucleotides may e.g. be used to introduce restriction sites to facilitate downstream cloning. Heavy and light chain PCR fragments may then e.g. be subcloned into suitable expression vectors (e.g. pCMV) and transfected into Expi293 cells (Life technologies) using Expi293-fection (Life Technologies) according to the manufacturers' instructions. The transfected cells may then e.g. grown for 7 days at 37°C in a 5% CO<sub>2</sub> environment using growth media that allow the production of the inventive antibody. Resultant supernatants may e.g. harvested after 5 to 7 days.

In one embodiment the inventive antibody or antigen-binding fragment thereof as is further coupled to a detectable label. As used for the inventive antibody or antigen-binding fragment thereof the term "detectable label" refers to a molecule capable of detection, which may e.g. include radioactive isotopes, fluorescent probes, chemiluminescences, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, dyes, metal ions, or biotin. The term "coupled" as used for the inventive antibody or antigen-binding fragment thereof refers to the fact that the dye, radioisotope may e.g. be non-covalently via e.g. ionic, or hydrophobic interactions, or covalently attached to inventive antibody or antigen-binding fragment thereof. Coupling of the detectable labels as disclosed above such as e.g. of fluorescent probes, dyes, or enzymes to the inventive antibody or antigen-binding fragment thereof as disclosed above may e.g. be done according to methods known in the art such as those disclosed in Methods Cell Biol. 2001;63:185-204; Methods Mol Biol. 2010;588:43-8; Curr Protoc Mol Biol. 2001 May;Chapter 11:Unit 11.1.

According to one embodiment the detectable label coupled to the inventive antibody or antigen-binding fragment thereof is one of an enzyme, or fluorophore, or enzyme substrate. For example, the detectable label coupled to the inventive antibody or antigen-binding fragment thereof may be alkaline phosphatase, horseradish peroxidase, beta-galactosidase, Tobacco Etch Virus nuclear-inclusion-a endopeptidase ("TEV protease"). The fluorophore which may e.g. be coupled to the inventive antibody as disclosed above may be one of 1,8-ANS, 4-methylumbelliferone, 7-amino-4-methylcoumarin, 7-hydroxy-4-methylcoumarin, Acridine, Alexa Fluor 350™, Alexa Fluor 405™, AMCA, AMCA-X, ATTO Rho6G, ATTO Rho11, ATTO Rho12, ATTO Rho13, ATTO Rho14, ATTO Rho101, Pacific Blue, Alexa Fluor 430™, Alexa Fluor 480™, Alexa Fluor 488™, BODIPY 492/515, Alexa Fluor 532™, Alexa Fluor 546™, Alexa Fluor 555™, Alexa Fluor 594™, BODIPY 505/515, Cy2, cyQUANT GR, FITC, Fluo-3, Fluo-4, GFP (EGFP), mHoneydew, Oregon Green™ 488, Oregon Green™ 514, EYFP, DsRed, DsRed2, dTomato, Cy3.5, Phycoerythrin (PE), Rhodamine Red, mTangerine, mStrawberry, mOrange, mBanana, Tetramethylrhodamine (TRITC), R-Phycoerythrin, ROX,

DyLight 594, Calcium Crimson, Alexa Fluor 594™, Alexa Fluor 610™, Texas Red, mCherry, mKate, Alexa Fluor 660™, Alexa Fluor 680™ allophycocyanin, DRAQ-5, carboxynaphthofluorescein, C7, DyLight 750, Cellvue NIR780, DM-NERF, Eosin, Erythrosin, Fluorescein, FAM, Hydroxycoumarin, IRDyes (IRD40, IRD 700, IRD 800), JOE, Lissamine rhodamine B, Marina Blue, Methoxy coumarin, Naphtho fluorescein, PyMPO, 5-carboxy-4',5'-dichloro-2',7'-dimethoxy fluorescein, 5-carboxy-2',4',5',7'-tetrachlorofluorescein, 5-carboxyfluorescein, 5-carboxyrhodamine, 6-carboxyrhodamine, 6-carboxytetramethyl amino, Cascade Blue, Cy2, Cy3, Cy5,6-FAM, dansyl chloride, HEX, 6-JOE, NBD (7-nitrobenz-2-oxa-1,3-diazole), Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, phthalic acid, terephthalic acid, isophthalic acid, cresyl fast violet, cresyl blue violet, brilliant cresyl blue, para-aminobenzoic acid, erythrosine, phthalocyanines, azomethines, cyanines, xanthenes, succinylfluoresceins, rare earth metal cryptates, europium trisbipyridine diamine, a europium cryptate or chelate, diamine, dicyanins, or La Jolla blue dye. Fluorophores which may be coupled to the inventive antibody or antigen-binding fragment may e.g. also include quantum dots. The term quantum dot as used in the present invention refers to a single spherical nanocrystal of semiconductor material where the radius of the nanocrystal is less than or equal to the size of the exciton Bohr radius for that semiconductor material (the value for the exciton Bohr radius can be calculated from data found in handbooks containing information on semiconductor properties, such as the CRC Handbook of Chemistry and Physics, 83rd ed., Lide, David R. (Editor), CRC Press, Boca Raton, Fla. (2002)). Quantum dots are known in the art, as they are described in references, such as Weller, *Angew. Chem. Int. Ed. Engl.* 32: 41-53 (1993), Alivisatos, *J. Phys. Chem.* 100: 13226-13239 (1996), and Alivisatos, *Science* 271: 933-937 (1996). Quantum dots may e.g. be from about 1 nm to about 1000 nm diameter, e.g. 10 nm, 20 nm, 30 nm, 40 nm, 50 nm, 60 nm, 70 nm, 80 nm, 90 nm, 100 nm, 150 nm, 200 nm, 250 nm, 300 nm, 350 nm, 400 nm, 450 nm, or 500 nm, preferably at least about 2 nm to about 50 nm, more preferably QDs are at least about 2 nm to about 20 nm in diameter (for example about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nm). QDs are characterized by their substantially uniform nanometer size, frequently exhibiting approximately a 10% to 15% polydispersion or range in size. A QD is capable of emitting electromagnetic radiation upon excitation (i.e., the QD is photoluminescent) and includes a "core" of one or more first semiconductor materials, and may be surrounded by a "shell" of a second semiconductor material. A QD core surrounded by a semiconductor shell is referred to as a "core/shell" QD. The surrounding "shell" material will preferably have a bandgap energy that is larger than the bandgap energy of the core material and may be chosen to have an atomic spacing close to that of the "core" substrate. The core and/or the shell can be a semiconductor material including, but not limited to, those of the groups II-VI (ZnS, ZnSe, ZnTe, US, CdSe, CdTe, HgS, HgSe, HgTe, MgS, MgSe, MgTe, CaS, CaSe, CaTe, SrS, SrSe,

SrTe, BaS, BaSe, BaTe, and the like) and III-V (GaN, GaP, GaAs, GaSb, InN, InP, InAs, InSb, and the like) and IV (Ge, Si, and the like) materials, PbS, PbSe, and an alloy or a mixture thereof. Preferred shell materials include ZnS. Quantum dots may e.g. be coupled to the inventive antibody or antigen-binding fragment thereof by any method known in the art such as the methods disclosed in Nanotechnology. 2011 Dec 9;22(49):494006; Colloids and Surfaces B: Biointerfaces 84 (2011) 360–368.

In one example the inventive antibody or antigen-binding fragment thereof may be coupled to a radioisotope such as  $^{47}\text{Ca}$ ,  $^{14}\text{C}$ ,  $^{137}\text{Cs}$ ,  $^{157}\text{Cr}$ ,  $^{57}\text{Co}$ ,  $^{60}\text{Co}$ ,  $^{67}\text{Cu}$ ,  $^{67}\text{Ga}$ ,  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{129}\text{I}$ ,  $^{131}\text{I}$ ,  $^{32}\text{P}$ ,  $^{75}\text{Se}$ ,  $^{85}\text{Sr}$ ,  $^{35}\text{S}$ ,  $^{201}\text{Th}$ ,  $^3\text{H}$ , preferably, the radioisotopes are incorporated into a further molecule, such as e.g. a chelator. Typical chelators that may be used according to the invention are for example DPTA, EDTA (Ethylenediamine-tetraacetic acid), EGTA (Ethyleneglycol-O, O'-bis(2-aminoethyl)-N, N, N', N'-tetraacetic acid, NTA (Nitrilotriacetic acid), HEDTA (N-(2-Hydroxyethyl)-ethylenediamine-N,N',N'-triacetic acid), DTPA (2-[Bis[2-bis(carboxymethyl)amino]-ethyl]amino]acetic acid), or DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid).

In one embodiment the inventive antibody or antigen-binding fragment thereof is for use in detecting the presence or expression of an epitope comprised in SEQ ID NO: 1 in a sample. For example, the inventive antibody or antigen-binding fragment thereof is for use in detecting the expression of an epitope comprised in the amino acid sequence according to SEQ ID NO: 1, whereby SEQ ID NO: 1 corresponds to an intracellular portion of human PD-L1 spanning amino acid residues 260-290 of GenBank accession no. AAH69381. The epitope comprised in the amino acid sequence according to SEQ ID NO: 1 (e.g. within the intracellular domain of human PD-L1) may e.g. be reliably and reproducibly detected through the use of the inventive antibody or antigen-binding fragment thereof by means of immunohistochemistry on e.g. FFPE tissue samples, or tumor tissue samples. The term “epitope” as used for the present invention which is comprised in SEQ ID NO: 1 (RLRKGRMMDVKKCGIQDTN SKKQSDTHLEET) refers to a portion of SEQ ID NO: 1 having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a rabbit. The epitope comprised in SEQ ID NO: 1 according to the invention may e.g. be a linear epitope, or a conformational epitope. A conformational epitope may e.g. be composed of discontinuous sections of the amino acid sequence of SEQ ID NO: 1 and may e.g. interact with the paratope of the inventive antibody, or antigen-binding fragment as disclosed above based on its 3-dimensional surface features and shape or tertiary structure of SEQ ID NO: 1. The epitope comprised in SEQ ID NO: 1 according to the invention may e.g. also be a linear epitope formed by a continuous sequence of amino acids of SEQ ID NO: 1 which interacts with the paratope of the inventive antibody or antigen-binding fragment thereof based on the primary amino acid sequence of SEQ ID NO: 1 (RLRKGRMMDVKKCGIQDTNSKK QSDTHLEET). Accordingly, the paratope of inventive



antibody or antigen-binding fragment thereof may e.g. bind to a linear epitope comprised in the amino acid sequence according SEQ ID NO: 1 and may e.g. have a length of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 to about 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 amino acids, or of about 2 to about 3, 4, 5, 6, 7, 8, 9, 10 amino acids, or e.g. of about 8 amino acids to about 11 amino acids

5 For example, the linear epitope which interacts with the paratope of the inventive antibody or antigen-binding fragment thereof may comprise, or may be comprised in an amino acid sequences according to SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 31, SEQ ID NO: 32, 10 SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56., SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64. SEQ ID NO: 65, 15 SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, 20 SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO, 106, SEQ ID NO: 107, SEQ ID NO: 108. For example, the inventive antibody or antigen-binding fragment thereof as disclosed 25 above may be used to detect the presence or expression of an epitope as disclosed above in a sample, which may e.g. be a biological sample such as a body fluid, or a blood sample. For example, the biological sample, such as body fluid, or blood sample may be subjected to flow cytometry, or e.g. FACS using the inventive antibody or antigen-binding fragment thereof to detect the absence or presence of an epitope comprised in SEQ ID NO: 1, or as comprised in 30 e.g. any of the amino acid sequences according to SEQ ID NO: 19 – SEQ ID NO: 108 of the invention. For example, cells obtained by a needle biopsy, may be blocked for 10 minutes at room temperature with FcR blocker (e.g. TruStain FcX™ (Biolegend, San Diego; or e.g. Human BD Fc Block™, BD Biosciences) in a 1:1000 dilution and then incubated with the inventive antibody or antigen-binding fragment thereof, whereby the inventive antibody or 35 antigen-binding fragment thereof may be coupled to a fluorescent probe, e.g. phycoerythrin [PE] in a dilution of about 1:50 to about 1: 500, e.g. from about 1:75, 1:100, 1:125, 1:150, 1:175, 1:200 to about 1:250, 1:275, 1:300, 1:350, 1:400, 1:450, or e.g. 1:25, 1:50, 1:100, 1:150,

1:200, 1:250, 1:300, 1:400, 1:500 in PBS + 2% serum for 15 minutes in the dark at room temperature. Cells may then e.g. be analyzed on an Attune flow cytometer (Life Technologies, Grand Island, NY) and the results evaluated using FlowJo 10.0 software (Tree Star, Inc., Ashland, OR). For example, the cells may additionally be counterstained with  
5 carboxyfluorescein diacetate succinimidyl ester (CFSE, Biolegend, San Diego, CA) at a final concentration of 5  $\mu$ M for 10 minutes at 37°C in darkness, followed by two washes with RPMI-1640 medium.

According to a preferred embodiment the inventive antibody or antigen-binding fragment thereof may be used to detect the presence or expression of an epitope comprised in SEQ ID  
10 NO: 1, e.g. comprised in a linear epitope in any one of the amino acid sequences according to SEQ ID NO: 19 - SEQ ID NO: 108, in a tissue sample, fixed tissue sample, or a formaldehyde-fixed paraffin-embedded (FFPE) tissue. The term "tissue sample" according to the invention may e.g. refer to single cells derived from a tumor, or tissue suspected to be a tumor, or at  
15 least about  $10^2$ ,  $10^3$ ,  $10^4$ ,  $10^5$ ,  $10^6$  or more cells derived from a tumor tissue or tissue suspected to be a tumor which may e.g. also comprise cultured tumor cells, e.g. as described in Trends Biotechnol. 2013 June ; 31(6): 347–354; Cancer Cell Culture: Methods and Protocols (Methods in Molecular Medicine); S.P. Langdon (Ed.), Humana Press, ISBN: 978-1588290793.

The cells or tissue may e.g. be obtained by way of non-invasive means, including, but not limited to, fine needle aspiration and needle biopsy, or, alternatively, by e.g. an invasive  
20 method, including, but not limited to, surgical biopsy. The methods of obtaining the tissue sample do not form part of the present invention. The term "fixed tissue sample" according to the invention refers to a tissue sample which has been subjected to chemical tissue fixation. For example, chemical fixation may include fixation with methanol, ethanol, acetone, methanol-acetone mix, buffered formalin solution, glutaraldehyde, or buffered paraformaldehyde. For  
25 example, fixation may include treatment (fixation) of the tissue sample as disclosed above with 10% saturated aqueous formaldehyde buffered to pH 6.8–7.2 with 100 mM phosphate buffer, or 10% neutral buffered formalin (NBF) with varying times at temperatures ranging from 4°–45°C, e.g. room temperature (20–25°C), or e.g. from about 5°C, 6°C, 7°C, 8°C, 9°C, 10°C to about 15°C, 17.5°C, 20°C, or from about 12°C, 15°C, 17.5°C, 20°C to about 25°C, 27°C, 30°C,  
30 35°C, 40°C, 45°C for varying amounts of time. For example, the tissue samples as disclosed above may be fixed for about 5 minutes to about 24 hours, or from about 10min, 15min, 30min, 45min, 60 min, 90min, 120min, 180min to about 15min, 30min, 45min, 1h, 4h, 5h, 6h, 7h, 8h, 9h, 10h, 11h, 12h, 14h, 16h, 18h, 20h, 24h, 48h. For example, fixed tissue samples may include tissue samples that were fixed by immersion fixation with 10 % NBF for about 18-24h.  
35 For example, a fixed tissue sample according to the invention may includes a tissue sample as disclosed above which has been fixed, or treated according to standard protocols available in the art, such as those provided on the website [www.ihcworld.com](http://www.ihcworld.com), e.g. as described in

Hopwood D. Fixatives and fixation: a review. Histochem J. 1969;1(4):323–60, or e.g. as described in “Immunohistochemical Staining Methods”, 5<sup>th</sup> edition (2009), published by DAKO North America, Inc. For example formaldehyde-fixed paraffin-embedded (FFPE) tissues may be obtained by the following procedure:

- 5 The tumor tissue as disclosed above may e.g. be placed in at least 10 volumes of buffered formalin (3.7% formaldehyde:10mM phosphate buffer, ph-7.4) or buffered paraformaldehyde (3.7% paraformaldehyde: 10mM phosphate buffer, ph-7.4), followed by incubation in the buffered formalin, or NBF, for varying amounts of time depending on the thickness of the tissue sample as disclosed above e.g. for a 1-2mm tissue sample, 2-3h at RT, for a 5-10mm thick  
10 tissue sample– 5h RT, or e.g. for a tissue sample >10mm in thickness 2-3h RT, or e.g. at 4°C overnight. Subsequently, the tissue may e.g. be rinsed 1-2X with PBS, and stored at 4°C in 70% ethanol in H<sub>2</sub>O and then placed in the tissue processing histology cassettes and labeled for later identification. The histology cassettes may then e.g. be subjected to paraffin embedding using a commercial tissue processor 70% ethanol for 1 hour;95% ethanol (95%  
15 ethanol/5% methanol) for 1 hour; first absolute ethanol for 1 hour ; second absolute ethanol 1½ hours; third absolute ethanol 1½ hours; fourth absolute ethanol 2 hour; first clearing agent (xylene or substitute) 1 hour; second first clearing agent (xylene or substitute) 1 hour; first wax (Paraplast X-tra) at 58°C for 1 hour; second wax (Paraplast X-tra) at 58°C 1 hour. The formalin-fixed paraffin-embedded tissue may then be placed in a mold for further processing.
- 20 In one embodiment inventive antibody or antigen-binding fragment thereof as disclosed above is used in the detection of an epitope comprised in the amino acid sequences according to SEQ ID NO: 1, e.g. as comprised in any of the amino acid sequences according to SEQ ID NO: 19 – SEQ ID NO: 108 as disclosed above in a tissue sample, fixed tissue sample, or FFPE tissue sample derived from a tumor, e.g. the tissue obtained from a tumor by means of a biopsy  
25 as disclosed above. The term “derived from a tumor” according to the invention may e.g. also include tumor cells obtained by needle aspiration, or e.g. cells tumor cells obtained by needle aspiration which have been cultivated.

According to one embodiment the detection of the epitope comprised in the amino acid sequence according to SEQ ID NO:1 is done by means of immunohistochemistry (IHC), flow  
30 cytometry, ELISA, or western blotting. For example, the tissue sample, or tumor tissue sample according to the invention, may be subjected to immunoblotting to detect the epitope comprised in SEQ ID NO: 1 using the antibody or antigen binding fragment thereof according to the invention as described in Current Protocols in Molecular Biology 10.8.1-10.8.28, July 2008.

- 35 If, for example, an ELISA is used for the detection of the epitope comprised in SEQ ID NO: 1 using the inventive antibody or antigen-binding fragment thereof, the ELISA may be done

according to standard protocols, such e.g. as disclosed in Current Protocols in Molecular Biology (1991) 11.2.1-11.2.22. Preferably, the epitope comprised in SEQ ID NO: 1 is detected by means of immunohistochemistry (IHC) on FFPE tissue samples using the inventive antibody, or an antigen binding fragment thereof (e.g. as primary antibody): For example, the

5 FFPE tumor tissue sample may be deparaffinized by placing dry paraffin sections on slides in a 60°C oven for 1 hr. Subsequently, the slides may e.g. be placed in Sakura staining racks and immersed in Tissue-Tek® staining dishes containing the following solutions: three times in xylene for 5 min each two times in 100% ethanol for at least 1 min each two times in 95% ethanol for at least 1 min each one time in 70% ethanol for at least 1 min. The slides may then

10 be gently rinsed with tap water for about 5 minutes. Depending on the tumor tissue the sample is derived from it may e.g. be required to block endogenous peroxidase activity by placing the slides in a 3% hydrogen peroxide solution for 10 minutes at room temperature, followed by a rinse with water. Subsequent antigen retrieval may e.g. be done in a water bath according to the following procedure: Slides may be placed in a Coplin jar with antigen retrieval solution

15 such as e.g. Target Retrieval Solution, enhanced citrate buffer solution (Dako, S1699 or S1700), and Target Retrieval Solution, high pH (Dako, S3308), or 0.05M citrate buffer, pH 6, or e.g. Tris EDTA buffer, pH 8. Slides may e.g. then be allowed to equilibrate to 75° to 95°C in a water bath and incubated for about 40 minutes. The slides may e.g. then be allowed to cool at room temperature for 20 min after which the solution is decanted and the slides may then

20 be placed in a staining dish containing TBS/0.6% Tween 20 for a minimum of 5 minutes. Antigen retrieval may e.g. also be done using a PT link pretreatment module (DAKO) using Tris-EDTA buffer pH 9 at 97°C for 20 minutes. Following the antigen retrieval the slides may then be subjected to the staining procedure using an automated instrument (e.g. Discovery XT®, or AutostainerLink 48) following the manufacturer's instructions. For example, the slides

25 may also be manually processed as described in Current Protocols in Molecular Biology 14.6.1-14.6.23, January 2008. For example, the slides may be covered with 400 to 500 µl of the antibody according to the invention diluted e.g. into commercially available antibody diluent (e.g. from DAKO) to a concentration of about 0.2µg/ml to about 5µg/ml, e.g. from about 0.2 µg/ml, 0.3µg/ml, 0.4µg/ml, 0.5µg/ml, 0.6µg/ml, 0.7 µg/ml, 0.8µg/ml, 0.9µg/ml, 1.0 µg/ml, 1.25

30 µg/ml, 1.5 µg/ml, 1.75 µg/ml, 2.0µg/ml to about 3µg/ml, 3.5µg/ml, 4µg/ml, 4.5 µg/ml, 5µg/ml, 5.5µg/ml, 6.0µg/ml, 7.0µg/ml, 8.0µg/ml, 9.0µg/ml, 10 µg/ml and incubated for about 30 minutes at room temperature in a moist chamber. The primary antibody may then be rinsed off with TBS/0.6% Tween 20®. The slides may e.g. then be gently drained and freed from any remaining wash solution. Immediately thereafter the secondary antibody for the detection of

35 the inventive antibody may be added and incubated at room temperature for about 30 min. Secondary antibody dilution may e.g. be from about 1:100 to about 1:10.000, e.g. from about 1:100, 1: 150, 1:200, 1:250, 1:300, 1:400, 1:500, 1:750, 1:1000 to about 1:1500, 1:2000,

1:2500, 1:3000, 1:3500, 1:4000, 1:5000, 1:5500, 1:6000, 1:7000, 1:8000, 1:9000, or e.g. from about 1:100, 1: 150, 1:200, 1:250, 1:300, 1:400, 1:500, 1:750 to about 1:1.000, 1:2000. Secondary antibodies that can e.g. be used for the detection of bound antibody according to the invention may include polyclonal goat anti-rabbit HRP-conjugated immunoglobulins at a dilution of about 1:50, 1:175, to about 1:200, or e.g. goat anti-rabbit alkaline phosphatase (AP)-conjugated immunoglobulins at a dilution of about 1:20, 1:50, 1:100 to about 1:100, 1:200, 1:250, depending on the choice of the detection method and substrate employed, e.g. if Horseradish peroxidase (HRP)-conjugated secondary antibodies are used, 3,3'-diaminobenzidine (DAB) may e.g. be used for chromogenic detection, or e.g. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), or e.g. 3,3',5,5'-Tetramethylbenzidine (TMB) may be used, or if e.g. AP-conjugated secondary antibodies are used, a substrate combination of nitro blue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) may be used. General principles and guidelines on chromogenic immunohistochemistry can e.g. be found in Current Protocols in Immunology 21.4.21-21.4.26, November 2013.

The slides may then be washed twice with TBS/0.6% Tween 20® by overlaying the slide with wash solution for about 5 minutes after which the chromogen solution may be added according to the manufacturer's recommended protocol and incubated for about 4 to 5 min. A counterstain may e.g. be made using Harris' hematoxylin for about 5 minutes after which the slides may be rinsed under running tap water followed by e.g. an additional rinse in TBS/0.6% Tween 20 for about 1 min. The sections may then e.g. be dehydrated by gently immersing the slides up and down for several seconds in each solution before transferring to the next: 70% ethanol 90% ethanol 100% ethanol two times in xylene. The sections may then e.g. be mounted using a mounting medium and coverslip.

In one aspect the present invention provides for a method of detecting the presence or expression of an epitope comprised in SEQ ID NO:1 in a sample, e.g. an epitope comprised in any of SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56., SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64. SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID

NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, wherein the method comprises the step of contacting a sample with an antibody or antigen binding fragment according to the invention, and detecting the presence of bound antibody or antigen binding fragment according to the invention. For example, the inventive method of detecting the presence or expression of an epitope comprised in SEQ ID NO:1 in a sample may comprise detection by means of immunohistochemistry, or by means of automated immunohistochemistry using commercially available IHC platforms, such as e.g. IntelliPath FLX (Biocare Medical), WAVE RPD (Celerus Diagnostics), Omnis (DAKO), Autostainer Link 48 (DAKO), Benchmark XT (Ventana), or Benchmark Ultra (Ventana) according to the corresponding manufacturer's instructions and/or protocols. Accordingly, the inventive method may be used to detect the expression of human PD-L1 in a sample according to the invention.

In one embodiment the inventive method may be used to detect the presence of human PD-L1 or any fragment thereof comprising the amino acid sequence according to SEQ ID NO: 1 in a sample, wherein the method comprises the step of contacting said sample with the inventive antibody or antigen binding fragment thereof and detecting the presence of bound antibody or antigen binding fragment thereof as disclosed above, e.g. by means of immunohistochemistry, or by means of automated immunohistochemistry. For example, fragments of human PD-L1 according to the invention may comprise human PD-L1 which has been proteolytically cleaved, whereby the cleaved PD-L1 fragment comprises the amino acid sequence according to SEQ ID NO: 1. For example, a fragment of human PD-L1 according to the invention may comprise human PD-L1 lacking the extracellular domain, e.g. only comprises the transmembrane domain and the intracellular domain (e.g. amino acids 239-290 of GenBank accession no. AAH69381), or e.g. PD-L1 fragments which may be detected using the inventive antibody may only comprise the intracellular domain of human PD-L1 (e.g. amino acids 260-290).

In one aspect, the inventive antibody or antigen-binding fragment thereof is used to detect the presence or expression of an epitope comprised in SEQ ID NO:1 in a sample as disclosed above, e.g. an epitope comprised in any of SEQ ID NO: 19 - SEQ ID NO: 108 as disclosed above. For example, the sample may be a biological sample, such as e.g. body fluid, or a blood sample. For example, the presence or expression of an epitope comprised in SEQ ID NO:1 in a blood sample may be done using the inventive antibody or antigen-binding fragment as disclosed above. The blood sample used may e.g. of mammalian origin, preferably a human blood sample, preferably a blood sample from a cancer patient, or from an individual at risk of

having cancer. In one aspect the inventive antibody or antigen-binding fragment thereof may be used to e.g. detect the presence or expression of PD-L1 expression on circulating tumor cells, or e.g. leukocytes, such as neutrophils, eosinophils, basophils, lymphocytes or monocytes.

5 For example, using flow cytometry a peripheral blood sample (e.g. from a tumor patient, whereby the term “tumor” refers to neoplasms, i.e. abnormal growth of tissue which may also form a mass ) may be analyzed using phycoerythrin-Texas Red (ECD)-conjugated anti-CD3, phycoerythrin-Cyanin 5 (PC5)-conjugated anti-CD15 (BD Biosciences, San Diego, CA, USA), fluorescein isothiocyanate (FITC)-conjugated anti-CD80 (MIH clones, eBioscience, San Diego,  
10 CA, USA) in conjunction with the inventive antibody or antigen-binding fragment thereof conjugated to phycoerythrin (PE) to identify the cell types expressing the epitope comprised in SEQ ID NO: 1. For example, neutrophils may be identified as CD15<sup>+</sup>CD3<sup>-</sup> cells, the labeling of which may e.g. be done by incubating 50 µL of fresh heparinized whole blood simultaneously with 5 µL ECD-conjugated anti-CD3, 5 µL PC5-conjugated anti-CD15 on ice in the dark for 30  
15 minutes. Cells incubated with PE- and FITC- conjugated mouse IgG may e.g. be used as isotype controls. The samples may then be analyzed using a cell sorter, e.g. a CYTOMICS FC 500 flow cytometer (Beckman Coulter Inc., Brea, CA, USA) and associated software programs (CXP) according to the manufacturer’s instructions.

The presence or expression of the epitope comprised in the amino acid sequence according to SEQ ID NO: 1, or e.g. comprised in any of the amino acid sequences according to SEQ ID  
20 NO: 19 – SEQ ID NO: 108, on circulating tumor cells (CTCs) using the inventive antibody or antigen-binding fragment thereof as disclosed above may e.g. be analyzed by flow cytometry as disclosed above using one or more of the following markers EpCAM, EphB4, EGFR, CEA, HER2, or MUC-1 in combination with the inventive antibody as disclosed above, whereby the  
25 one or more antibodies used to label the CTCs has a different fluorescent label than the inventive antibody as disclosed above. CTCs may e.g. be isolated from a blood sample, e.g. a human blood sample as disclosed above, by commercially available isolation methods such as CellSearch, or a CTC Chip using e.g. ClearCell®FX CTC-based enrichment, e.g. according to the manufacturer’s instructions. In the above methods, the inventive antibody or antigen-  
30 binding fragment thereof conjugated to a fluorescent probe or detectable label as disclosed above may e.g. be used in a concentration from about 0.01 µg/ml to about 50 µg/ml, e.g. from about 0.025 µg/ml, 0.05 µg/ml, 0.075 µg/ml, 0.1 mg/ml, 0.25 µg/ml, 0.5 µg/ml, 0.75 µg/ml, 1 µg/ml, 1.50 µg/ml, 1.75 µg/ml, 2 µg/ml, 2.5 µg/ml, 3 µg/ml, 3.5 µg/ml, 4 µg/ml, 4.5 µg/ml, 5 µg/ml, 5.5 µg/ml, 6 µg/ml, 7 µg/ml, 8 µg/ml, 9 µg/ml, 10 µg/ml to about 11 µg/ml, 12 µg/ml, 15 µg/ml,  
35 17.5 µg/ml, 20 µg/ml, 22.5 µg/ml, 25 µg/ml, 30 µg/ml, 35 µg/ml, 37.5 µg/ml, 40 µg/ml, 45 µg/ml, 50 µg/ml, or e.g. from about 0.5 µg/ml, 0.75 µg/ml, 1 µg/ml, 1.50 µg/ml, 1.75 µg/ml, 2 µg/ml, 2.5 µg/ml, 3 µg/ml, 3.5 µg/ml, 4 µg/ml, 4.5 µg/ml, 5 µg/ml to about 6 µg/ml, 7 µg/ml, 8 µg/ml, 9 µg/ml,

10µg/ml, or from about 0.5µg/ml, 0.75µg/ml to about 1µg/ml, 1.50µg/ml, 1.75 µg/ml, 2µg/ml, 2.5µg/ml, 3µg/ml, 3.5µg/ml, 4µg/ml, 4.5 µg/ml, 5µg/ml, 5.5µg/ml, 6µg/ml, 7µg/ml, 8µg/ml, 9µg/ml, 10µg/ml, or e.g. at a concentration of 0.5µg/ml, 0.75µg/ml, 1µg/ml, 1.50µg/ml, 1.75 µg/ml, 2µg/ml, 2.5µg/ml, 3µg/ml, 3.5µg/ml, 4µg/ml, 4.5 µg/ml, 5µg/ml, 5.5µg/ml, 6µg/ml, 7µg/ml, 8µg/ml, 9µg/ml, 10µg/ml.

For example, in one aspect of the invention the presence or expression of an epitope comprised in the amino acid sequence according to SEQ ID NO: 1, or e.g. in any of the amino acid sequences according to SEQ ID NO: 19 – SEQ ID NO: 108, using the inventive antibody or antigen-binding fragment in a sample, such as a blood sample may be done by means of an ELISA. For example, the inventive antibody may be used at a concentration of about 0.25µg/ml, 0.5µg/ml, 0.75µg/ml, 1µg/ml, 1.50µg/ml, 1.75 µg/ml, 2µg/ml, 2.5µg/ml, 3µg/ml, 3.5µg/ml, 4µg/ml, 4.5 µg/ml, 5µg/ml, 5.5µg/ml, 6µg/ml, 7µg/ml, 8µg/ml, 9µg/ml, 10µg/ml, or a control antibody (e.g. mIgG2a at 2 µg/ml in PBS) to coat an ELISA plate overnight at 4°C followed by blocking with PBS containing 10% FBS. Blood samples (e.g. from a cancer patient, and/ from healthy individual as control or reference sample) may e.g. be diluted in PBS at 1:1.000 in triplicate before adding to the plates. Subsequently, the wells may e.g. be washed six times in PBS with 0.1% Tween-20. Bound inventive antibody may be detected by horseradish peroxidase-conjugated secondary anti-rabbit IgG Ab at a 1:2.000 dilution incubated for 1.5 hour at room temperature and then reacted with tetramethylbenzidine followed by a measuring absorbance using a plate reader at a wavelength of 450 nm. Nonspecific binding of sera to plates coated with control Ig may e.g. be subtracted from the measurement of each sample for background correction.

According to a preferred embodiment the inventive antibody or antigen-binding fragment thereof may be used to detect the presence or expression of an epitope comprised in SEQ ID NO: 1, or e.g. in any of the amino acid sequences according to SEQ ID NO: 19 – SEQ ID NO: 108, in a tissue sample, fixed tissue sample, or a formaldehyde-fixed paraffin-embedded (FFPE) tissue as disclosed above. Preferably, according to one embodiment the formaldehyde-fixed paraffin-embedded (FFPE) tissue is a tumor tissue sample, or a tumor tissue-derived sample, e.g. tumor cells, cultured tumor cell lines, or tumor tissue which has been cultured as disclosed above. The inventive method may e.g. in one embodiment also comprise subjecting control tissue which does not express or only minor amounts of PD-L1 to the inventive method, preferably processed in parallel to the tissue sample as disclosed above. For example, the control tissue may comprise thyroid tissue or skeletal muscle tissue, or cultured PD-L1 negative cancer cells, such as e.g. A2780, Colo205 or IGROV-1. The term minor amounts as used above refers to the expression of PD-L1 by a tumor cell or tumor tissue which is at least 5-, 10-, 20-, 25-, 40-, 50-, or 100-fold less than that of a PD-L1 positive tumor tissue or tumor cell (such as e.g. Hs746T, MDA-MB 231, NCI-H2009, human spleen) when



compared by semiquantitative immunoblotting, or qPCR as described in e.g. Journal of Immunological Methods 345 (2009) 40–48; Journal of Immunological Methods 353 (2010) 148–150; Hematology. 2016 Mar 31:1-6. For example, the tissue sample, or tumor tissue sample as defined above may be subjected to RNA isolation and cDNA synthesis which may then e.g. be used in qPCR to determine the relative expression level of PD-L1.

In one aspect the inventive antibody may e.g. be used on tissue samples mounted on a glass slide to form a tissue array in which different tumor tissue samples may be placed next to each other. For example, a tissue array comprises at least one, two, three or four sections of each tissue sample mounted on a glass slide in different locations (e.g. to avoid detection artefacts).

For example, the tissue array may comprise at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 18, 20 to about 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 55 or 60, or from about 22, 24, 28, 30, 36, 40, 44, 48, 52, 56, 60 to about 64, 68, 72, 76, 78, 80, 82, 84, 86, 90, 100 different tissue samples. For example, in one embodiment the tissue array may comprise at least one or more, e.g. 5, 10, 20, 30, 40, 50 or more, or e.g. all of the cancer cell lines ZR-75-1, WM164, U-87MG, U-118MG, T47D, SW707, SW620, SNB-78, NCI-H69, NCI-H569, NCI-H460, NCI-H292, NCI-H2009, NCI-H1975, Mx-1, MeWo, PC-3, Raji, Ramos, suit7, MDA-MB-468, MDA-MB-435, MDA-MB-231, MCF7, M24met, Lox, LoVo, Kyse 30, Jurkat, IGROV-1, HT29, Hs746T, Hs68, HL60, FaDu, EBC-1, DU 145, DLD-1, COLO 205, Calu 6, Calu 3, BT474, AGS, A549, A-431, A2780, Pfeiffer, MiaPaCa-2, ME-SA, in addition to the tumor tissue sample as disclosed above. The cancer cell lines as disclosed above may e.g. be obtained from ATCC, or e.g. DSMZ (Leipzig Institute DSMZ) and cultured according to established methods for each of the respective cell lines. Tissue arrays or tumor tissue arrays comprising at least one formaldehyde-fixed paraffin-embedded (FFPE) tissue sample, or FFPE tumor tissue sample as disclosed above may e.g. be manufactured according to the protocol as described in Nat Med. 1998 Jul;4(7):844-7.

According to one embodiment, the formaldehyde-fixed paraffin-embedded (FFPE) tissue sample, or formaldehyde-fixed paraffin-embedded tumor tissue sample is derived from a subject being at risk of, or having cancer, T cell dysfunction, acute or chronic infection or showing tumor immunity. The term cancer as used for the inventive method refers to or describes the physiological condition in mammals, preferably in humans, that is typically characterized by unregulated cell growth/proliferation with the potential to invade or spread to other parts of the body. Examples of cancer include, but are not limited to, non-small-cell lung cancer (NSCLC), mesothelioma, unresectable mesothelioma, breast cancer, adenocarcinoma of stomach or GEJ, gastric, Thymoma, ovarian cancer, adenoid cystic carcinoma, metastatic adenoid cystic carcinoma, bladder cancer, clear cell kidney cancer, head/neck squamous cell carcinoma, lung squamous cell carcinoma, malignant melanoma, ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, small-cell lung cancer (SCLC) or triple negative

breast cancer, lymphoproliferative disorders, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), diffuse large B-cell lymphoma (DLBCL), EBV-positive DLBCL, primary mediastinal large B-cell lymphoma, T- cell/histiocyte-rich large B-cell lymphoma, follicular lymphoma, Hodgkin's lymphoma (HL), mantle cell lymphoma (MCL), multiple myeloma (MM), myeloid cell leukemia-1 protein (Mcl-1), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or small lymphocytic lymphoma (SLL), Merkel cell carcinoma (MCC), or squamous head and neck cancer (SHNC). The above cancer types may e.g. also be referred to as malignancy. The term "T cell dysfunction" as used for the inventive method as disclosed above refers to a pathological state of CD8 T cells that e.g. can be caused if alterations in the tightly controlled differentiation process of a CD8 positive T cell occurs, such as e.g. changes in the nature, context and duration of antigen encounter which can result in substantial alterations in T cell activation and differentiation process that may also be referred to as T-cell exhaustion, tolerance, anergy or senescence. The term acute infection as used for the inventive method refers to an acute infection caused by bacteria, viruses or parasites and which e.g. has occurred about one, two, three, or four weeks prior to diagnosis. The term "chronic infection" as used for the inventive method refers to a situation in which pathogens are not quickly eliminated but rather persist for an extended period of time, e.g. for more than three, four, five, six, seven, eight, nine or ten weeks. The persistent pathogen exposure can result in chronic antigen stimulation and persistent inflammation which can result in exhaustion and/or clonal depletion of pathogen-specific CD8 T cells. Chronic infections may e.g. include viral infection such as hepatitis A, hepatitis C or hepatitis D, HIV infections, or e.g. infections with neisseria meningitidis, neisseria gonorrhoeae, bartonella henselae, Borrelia burgdorferi, salmonella species, brucella species campylobacter species, mycobacteria species, treponema pallidum, or coxiella burnetii, if left untreated. In one embodiment the FFPE tissue sample, or FFPE tumor tissue sample is derived from a subject, e.g. a human, characterized by or having tumor immunity. The term "tumor immunity" as used for the inventive method refers to the process in which tumors evade immune recognition and clearance. Tumor-derived, or tumor-associated factors are believed to influence dendritic cell differentiation and preclude the development of cells with antigen-presenting function that ultimately result in tumor immunity. The expression of endogenous retroviruses (ERVs) in particular tumor-specific endogenous retroviruses (TERVs) has e.g. also been implicated in tumor immunity. Other factors that may contribute to tumor immunity are e.g. gene amplifications PD-L1(CD274) and ALOX12B/15B, or e.g. mutations on any of the genes B2M, HLA-A, HLA-B, HLA-C, or CASP8 that result in a loss of antigen presentation (e.g. of tumor associated neoantigens), or result in blockage of extrinsic apoptosis.

In one aspect the present invention provides for a method of predicting that a mammal, preferably a human, suffering from a malignancy is a likely candidate for treatment with an immune check-point inhibitor, such as e.g. an anti-PD-L1 antibody, or an anti-PD-1 antibody which comprises detecting the presence or absence of an epitope comprised in SEQ NO: 1, or e.g. comprised in any of SEQ ID NO: 19 - SEQ ID NO: 108, e.g. SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, in a sample derived from a mammal, e.g. from an individual in need thereof, e.g. a human, having or at risk of cancer, T cell dysfunction, acute or chronic infection or tumor immunity, or e.g. a cancer patient suffering from a malignancy, whereby the method comprises e.g. flow cytometry, FACS, or preferably immunohistochemistry using the inventive antibody, wherein detecting the epitope comprised in SEQ ID NO: 1, or e.g. comprised in any of SEQ ID NO: 19 to SEQ ID NO: 108, in e.g. more than about 0.25%, 0.5%, 0.75%, 1%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%, 4.5%, 5.0%, 6.0%, 6.5%, 7.0%, 7.5%, 8.0%, 8.5%, 9.0%, 9.5%, or 10.0%, or in about 0.5% 0.75%, 1%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%, 4.5%, 5.0%, 6.0%, 6.5%, 7.0%, 7.5%, 8.0%, 8.5%, 9%, 9.5%, 10%, 10.5%, 11%, 12%, 13%, 14%, 15% to less than about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or e.g. from about 20%, 25%, 30%, 35%, 40%, 45% to less than about 65%, 70%, 75%, 80%, 85%, 90%, 95%, or from about 0.25%, 0.5%, 0.75%, 1% to less than about 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%, 4.5%, 5.0%, 6.0%, 6.5%, 7.0%, 7.5%, 8.0%, 8.5%, 9.0%, 9.5%, or 10.0%, or from about 0.25% to less than about 0.5%, 0.75%, 1% of the cells in the sample indicates that the patient is likely to respond to a treatment with an immune checkpoint-inhibitor, e.g. to a treatment with an anti-PD-L1 antibody, or to a treatment with an anti-PD-1 antibody. The term "immune checkpoint inhibitor", as used herein

has its general meaning in the art and refers to a molecule that is expressed by T cells in that either turn up a signal (stimulatory checkpoint molecules) or turn down a signal (inhibitory checkpoint molecules), such as for example the PD-1/PD-L1 and CTLA-4/CD28 signaling pathways both of which are recognized in the art to constitute immune checkpoint pathways (see e.g. Pardoll, 2012. Nature Rev Cancer 12:252-264). Anti-PD-L1 antibodies that may e.g. be administered to patients that have been identified as likely candidates for the treatment with an immune checkpoint inhibitor using the inventive method as disclosed above include e.g. avelumab, durvalumab, atezolizumab, or e.g. anti-PD-1 antibodies such as pembrolizumab, nivolumab, or Pf-06801591.

PD-L1 positive cells in the sample according to the invention may e.g. include tumor cells, or immune cells, such as tumor infiltrating lymphocytes (TILs) (e.g. CD8-positive T cells, macrophages, NK cells, or B-cells). For example, depending on the tumor type from which the FFPE tumor tissue sample as disclosed above was obtained or derived, different methods for determining the relative abundance of PD-L1 positive cells in a sample (scoring method) may be used. For example, using immunohistochemistry (IHC) as disclosed above using the inventive antibody to detect PD-L1 positive cells, PD-L1 scoring may include determining the relative abundance of PD-L1 positive tumor cells, or the relative abundance of PD-L1 positive TILs in the sample, or e.g. determining the combined relative abundance of PD-L1 positive tumor cells and TILs. The total number of cells in the sample, or any subfraction thereof which is used for determining the number of PD-L1 positive cells, may e.g. be obtained by counterstaining the sample using hematoxylin to visualize cell nuclei and cytoplasm, or e.g. using a 4',6-Diamidino-2-phenylindole (DAPI) stain to visualize cell nuclei if fluorescent detection methods are used. The term "PD-L1 positive cells" as used for the inventive method refers to cells which detectably express an epitope comprised in the amino acid sequence according to SEQ ID NO: 1 to which the inventive antibody specifically binds and which then may be detected with e.g. HRP- or AP-coupled secondary antibodies which specifically bind to the inventive antibody and allow chromogenic or fluorescent detection when reacted with the corresponding substrate (e.g. as disclosed above). For example, HRP-coupled secondary antibodies may be used and incubated with DAB as substrate to label PD-L1 positive cells. The scoring of PD-L1 positive cells may e.g. include counting individual cells or nuclei, to determine the number of PD-L1 positive cells and total cell number in the FFPE tumor tissue sample analyzed (or e.g. in any subsection thereof). Alternatively, the surface area of PD-L1 positive cells and hematoxylin stained cells may e.g. be used to determine the relative abundance of PD-L1 positive cells.

In one aspect the inventive method as disclosed above may be used to identify likely candidates for a treatment with an anti-PD-L1 antibody whereby the malignancy according to the invention may be one of non-small-cell lung cancer (NSCLC), mesothelioma, unresectable

mesothelioma, breast cancer, adenocarcinoma of stomach or GEJ, gastric, Thymoma, ovarian cancer, adenoid cystic carcinoma, metastatic adenoid cystic carcinoma, bladder cancer, clear cell kidney cancer, head/neck squamous cell carcinoma, lung squamous cell carcinoma, malignant melanoma, ovarian cancer, pancreatic cancer, prostate cancer, renal cell cancer, small-cell lung cancer (SCLC) or triple negative breast cancer, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), diffuse large B-cell lymphoma (DLBCL), EBV-positive DLBCL, primary mediastinal large B-cell lymphoma, T- cell/histiocyte-rich large B-cell lymphoma, follicular lymphoma, Hodgkin's lymphoma (HL), mantle cell lymphoma (MCL), multiple myeloma (MM), myeloid cell leukemia-1 protein (Mcl-1), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or small lymphocytic lymphoma (SLL), Merkel cell carcinoma (MCC), or squamous head and neck cancer (SHNC).

According to one aspect of the present invention the inventive method may be used to identify or predict likely candidates (e.g. cancer patients who will respond to a treatment with an immune checkpoint inhibitor, such as an anti-PD-L1 antibody, or anti-PD-1 antibody with one of the following anti-PD-L1 antibodies avelumab, atezolizumab, durvalumab, LY300054, BMS-936559, or anti-PD-1 antibodies pembrolizumab, nivolumab, or Pf-06801591. For example, if an individual in need thereof, e.g. a human cancer patient, or e.g. a human patient suffering from a malignancy as disclosed above, or a human at risk of having cancer, by applying the above inventive method is found to be a likely candidate for the treatment with an anti-PD-L1 antibody, e.g. one of avelumab, atezolizumab, durvalumab, LY300054, BMS-936559, or with an anti-PD-1 antibody, e.g. one of pembrolizumab, nivolumab, or Pf-06801591 may be administered alone or in combination with other anti-cancer agents in a dose from about 0.1 mg/kg to about 50 mg/kg, from about 0.5 mg/kg, 0.75 mg/kg, 1 mg/kg, 1.5 mg/kg, 2.5 mg/kg, 5 mg/kg, 7.5 mg/kg, 10 mg/kg, to about 12.5 mg/kg, 15 mg/kg, 17.5 mg/kg, 20 mg/kg, 25 mg/kg, 27.5 mg/kg, 30 mg/kg, 35 mg/kg, 37.5 mg/kg, 40 mg/kg, 42.5 mg/kg, 45 mg/kg, or e.g. 1 mg/kg, 2.5 mg/kg, 5 mg/kg, 7.5 mg/kg, 10 mg/kg, 12.5 mg/kg, 15 mg/kg, 17.5 mg/kg, 20 mg/kg, 22.5 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg. In one embodiment the anti-PD-L1 antibodies avelumab, atezolizumab, durvalumab, or e.g. the anti PD-1 antibodies pembrolizumab, nivolumab, or Pf-06801591 may e.g. be administered to likely candidates which have been identified by applying the inventive method as disclosed above in a flat dosing regimen, e.g. avelumab may be administered at flat dosing regimen of 500-800 mg every week, e.g. from about 600 mg – 750 mg every week, or e.g. from about 650-850 mg every week, or e.g. from about 700 – 900 mg every week, or e.g. 525 mg every week, 550 mg every week, 600 mg every week, 625 mg every week, 650mg every week, 700 mg every week, 725 mg every week, 750 mg every week, or e.g. from about 900 to about 1600 mg every two weeks, e.g. from about 1000 mg to about 1500 mg every two weeks (q2w), or from about 1250 mg to

about 1400 mg q2w, or from about 1000 mg q2w to about 1250 mg q2w, e.g. 925 mg q2w, 950 mg q2w, 1000 mg q2w, 1125 mg q2w, 1200 mg q2w, 1250 mg q2w, 1300 mg q2w, 1350 mg q2w, 1400 mg q2w, 1450 mg q2w, 1500 mg q2w, 1550 mg q2w, or e.g. from about 1250 – 2400 mg every three weeks (q3w), or from about 1500 mg to about 2250 mg q3w, or from about 1750 mg to about 2000 mg q3w, or from about 1800 mg to about 2300 mg q3w, or from about 1250 mg to about 1750 mg q3w, or from about 1300 mg to about 1500 mg q3w, or e.g. 150 mg q3w, 1350 mg q3w, 1450 mg q3w, 1550 mg q3w, 1650 mg q3w, 1750 mg q3w, 1800 mg q3w, 1850 mg q3w, 1900 mg q3w, 1950 mg q3w, 2000 mg q3w, 2125 mg q3w, 2250 mg q3w, 2350 mg q3w. The term “mg/kg” as used above refers to milligrams of anti-PD-L1 or anti-PD-1 antibody per kilogram body weight of a patient that will be subject to treatment with the respective antibody. The terms “q2w” and “q3w” as used herein shall indicate an administration every two weeks, or every three weeks. The term “mg/kg” as used above refers to milligrams of anti-PD-L1 (or e.g. anti-PD-1) antibody per kilogram body weight.

According to one aspect the present invention pertains to the use of the inventive antibody or antigen binding fragment thereof in the inventive method for predicting that a patient suffering from a malignancy is a likely candidate for treatment with an anti-PD-L1 antibody, or anti-PD-1 antibody. For example, the inventive antibody or antigen-binding fragment may be used in immunohistochemistry to detect PD-L1 expression in the FFPE tumor tissue sample and subsequently subjecting the sample to visual assessment of PD-L1 expression (scoring) to determine the number of PD-L1 positive cells, e.g. tumor cells, or TILs as disclosed above. The inventive antibody or antigen-binding fragment thereof may e.g. also be used in dual-color immunofluorescence to label TILs. For example dual color fluorescence may include the inventive antibody and one of an anti-CD8 antibody, anti-CD163 antibody, anti-CD3 antibody, anti-CD11c antibody, anti-CD56 antibody, anti-CD68 antibody, anti-Granzyme B antibody, or anti-FoxP3 antibody, whereby the antibodies are derived from different species to allow detection of both, the inventive antibody and of the TIL-specific antibody. Dual immunofluorescence may e.g. be carried out utilizing commercial kits, such as Novocastra PowerVision Poly-HRP IHC detection system and subsequent use of Alexa Fluor 594-, or Alexa Fluor 488 Tyramide Signal Amplification (TSA) Kit according to the manufacturer's instructions. The scoring of PD-L1 positive cells may e.g. be done as disclosed above, or may e.g. include assigning IHC scores based on PD-L1 positive tumor cells, PD-L1 positive immune cells (e.g. TILs, macrophages), or the aggregate score of PD-L1 positive tumor and immune cells e.g. with IHC scores of 0, 1, 2, or 3 corresponding to < 1% PD-L1 positive cells (IHC score of 0), >1%, but <5% (IHC score of 1), >5%, but <10% (IHC score of 2), or >10% (IHC score of 3). For example, if more than one sample of a given patient is subjected to the inventive in vitro method as disclosed above using the inventive anti-PD-L1 antibody as disclosed above, the IHC score may be the average score of the samples analyzed, or e.g. the IHC score may

correspond to the IHC score of the sample that has the highest IHC score. The IHC score may e.g. also be determined by scoring both tumor cells expressing PD-L1 using the inventive antibody or antigen-binding fragment thereof as a percentage of total tumor cells and tumor-infiltrating immune cells expressing PD-L1 as a percentage of tumor area. Samples may e.g. be scored 3 if  $\geq 50\%$  tumor cells were PD-L1 positive; score 2 if  $\geq 5\%$  but  $< 50\%$  tumor cells were PD-L1 positive; score 1 if  $\geq 1\%$  but  $< 5\%$  of tumor cells were PD-L1 positive; and score 0 if  $< 1\%$  tumor cells expressed PD-L1.

In one embodiment the present invention provides for a method of treatment of cancer in a patient who has been identified as a likely candidate to respond to the treatment with an immune checkpoint inhibitor by applying the inventive method as disclosed above to a sample obtained or derived from said patient and determining PD-L1 expression in that sample and in a second step comparing the expression of PD-L1 in that sample to the PD-L1 expression in a reference sample, and in a third step administering an immune checkpoint inhibitor, such as an anti-PD-L1, or anti-PD-1 antibody to said patient if the PD-L1 expression in the sample of said patient is found to be increased or above a certain threshold. The term "threshold" as used in the inventive method of treatment e.g. refers to a relative abundance of PD-L1 expression in the patient-derived sample compared to that of the reference sample as determined by the inventive method as disclosed above. For example, the threshold may be defined as the relative increase in PD-L1 positive cells in the patient sample compared to the reference sample, or e.g. may be defined as an absolute or relative number of PD-L1 positive cells in said sample, e.g. 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 40%, 50%, 75%, 80%,  $>1\%$ ,  $>5\%$ ,  $>10\%$ ,  $>20\%$ ,  $>50\%$ ,  $>75\%$ ,  $>80\%$  of the cells in the sample, or e.g. the threshold may be based on IHC score or scoring method as disclosed above. The reference sample used in the inventive method of treatment may e.g. be derived from a healthy donor who is not inflicted with cancer and which is subjected to the same method of detection of PD-L1 expression using the inventive antibody or antigen-binding fragment as disclosed above, such as e.g. IHC on FFPE tissue samples. For example, the reference sample may be obtained from the corresponding tissue and location as the sample obtained from the patient and is preferably processed in parallel to the sample from said patient. The reference sample may e.g. may also be derived from the same tissue or organ as the tumor tissue sample if it is obtained from an area or location that is not diseased., or e.g. the reference sample may be derived from a contralateral healthy organ. Anti-PD-L1 that may e.g. be administered to a patient according to the inventive method of treatment include avelumab, atezolizumab, durvalumab, or e.g. anti-PD-1 antibodies such as pembrolizumab, nivolumab, or Pf-06801591. In the inventive method of treatment the anti-PD-L1 or anti-PD-1 antibodies, such as e.g. avelumab, are administered as disclosed above, e.g. in a dose of from about 5mg/kg to about 30 mg/kg, or e.g. in a dose of 5 mg/kg, 7.5 mg/kg, 10 mg/kg, to about 12.5 mg/kg, 15 mg/kg,

17.5 mg/kg, 20 mg/kg, 25 mg/kg, 27.5 mg/kg, 30 mg/kg, whereby the antibody is administered once a week (q1w). The anti-PD-L1 antibody (e.g. avelumab) may also be administered as a flat dose every week, or every second week, or every three weeks as disclosed above, e.g. in a flat dose of 500-800 mg every week, or 900-1600 mg every two weeks, or 1250 – 2400 mg every three weeks.

In one embodiment the present invention provides an isolated polynucleotide encoding the antibody or antigen binding fragment thereof. The term "isolated polynucleotide" as used for the present invention refers to a polynucleotide which is at least 70%, 75%, 80%, 85%, 90%, preferably 95%, 96%, 97%, 98%, 99% pure and free of contaminants, such as e.g. proteins and/or lipids. The inventive antibody or antigen-binding fragment thereof may e.g. be encoded by two isolated polynucleotides encoding the light chain and the heavy chain of the inventive antibody. The isolated polynucleotides may e.g. comprise one polynucleotide sequence according to SEQ ID NO: 17 encoding the light chain of the inventive antibody and one polynucleotide comprising the polynucleotide sequence according to SEQ ID NO: 18 encoding the heavy chain of the inventive antibody. Depending on the expression system used the polynucleotide sequences according to SEQ ID NO: 17 and SEQ ID NO: 18 may be codon optimized to allow for a better expression of the inventive polynucleotides resulting in higher yields of the inventive antibody in a respective expression system. Codon optimization may e.g. be done using available codon usage tables as published in Nucleic Acids Res. 1988 Mar 11;16(5):1715-28, Nucleic Acids Res. 1988 Sep 12;16(17):8207-11; Methods Cell Biol. 1991;36:675-7; or e.g. available computer-embedded codon optimization tools such as those published in e.g. Bioinformatics. 2014 Aug 1;30(15):2210-2. Codon optimization of the at least one inventive polynucleotide may be done may in consideration of the respective expression system used, such as e.g. murine or human cell lines, yeast or insect cell lines.

In one embodiment the present invention provides for an expression vector comprising the inventive polynucleotide as disclosed above. The term "expression vector" according to the invention refers to nucleic acid vector that comprises a gene expression control sequence, such as e.g. a promoter or promoter component, operably linked in 5' to 3' direction to a nucleotide sequence encoding at least one polypeptide, e.g. SEQ ID NO: 17 and/or SEQ ID NO: 18. For example, the expression vector according to the invention may comprise the polynucleotide according to SEQ ID NO: 17, or SEQ ID NO: 18, or may comprise both polynucleotides according to SEQ ID NO: 17 and SEQ ID NO: 18. Nucleic acid sequences necessary for expression in prokaryotes include a promoter, a ribosome binding site, optionally an operator sequence and possibly other sequences. Eukaryotic cells utilize promoters, such as e.g. a CMV promoter, SV40 promoter, EF1a, or CAG promoter and often enhancers and polyadenylation signals. Exemplary mammalian expression vectors are pCMV, pCMV-SPORT, pcDNA, or e.g. the plasmid-based multigene expression system as described in Nat



Commun. 2010 Nov 16;1:120, or e.g. if viral expression of the inventive antibody or antigen-binding fragment thereof is intended, pLKO.1-hygro, pBABE-hygro, pBABE-puro, pBABE-zeo, pLenti CMV/TO zeo DEST, pCW57.1. Optionally, the expression vector in eukaryotes may comprise an internal ribosomal entry site (IRES) between the inventive polynucleotide sequences providing for a bicistronic expression construct to allow expression of both polynucleotides according to SEQ ID NO: 17 and SEQ ID NO: 18 from one expression vector. IRES sequences that e.g. may be used may include those published in PLoS One. 2013 Dec 9;8(12):e82100, e.g. 5' AUGAUAAUAUGGCCACAACCAUG. Suitable expression vectors that may e.g. be used for the expression of the inventive polynucleotides may comprise pCMV-based expression vectors, or pD912-based vectors and variants thereof, Gateway® expression vectors, pcDNA-based expression vectors, or pJΩ vectors (see e.g. Nucleic Acids Research, Vol. 18, No. 4), or vectors which may be used for retroviral or lentiviral production (see e.g. Front Biosci. 1999 Jun 1;4:D481-96.). Suitable yeast expression vectors may e.g. utilize GAL4, PGK, ADH1, ADE2 or TRP1 promoters for the expression of the inventive polynucleotides, e.g. expression vectors pRS420, pLEX, pACT2.2, pTEF1/zeo, pAG425GDP-ccdb, pBEVY-T, pAG300, pGBK T7, pEZY202, pCETT, pRG201, pXP120, pXP320, p2GLex, or p426 GAL. For example, the inventive polynucleotides as disclosed above may e.g. also be expressed in insect cells using expression vectors comprising a polyhedrin promoter such as e.g. pFastBac, pFastBac DUAL, pVL 1392, pVL 1393, or AcNPV for use in baculoviral expression of the inventive polynucleotides. In a preferred aspect the expression vector comprising the inventive polynucleotide is a mammalian expression vector.

In one embodiment the present invention pertains to the use of an expression vector according to the invention in producing the inventive antibody comprising the amino acid sequences according to SEQ ID NO: 15 and SEQ ID NO: 16. For example, the at least one vector according to the invention which comprises polynucleotides encoding the amino acid sequences according to SEQ ID NO: 15 and SEQ ID NO: 16 may be used in a transient expression system to express the inventive antibody. For example the transient expression system as disclosed in Methods. 2014 Jan 1;65(1):5-10 may be used. The inventive expression vector or at least one expression vector according to the invention may e.g. also be used for the generation of stable cell lines expressing the inventive antibody. For example, the process for development of a stable cell line may e.g. begin with transfection of a suitable host cell with the inventive expression vector, or with at least one expression vectors of the invention, e.g. two expression vectors, of which a first vector comprises the polynucleotides according to SEQ ID NO: 17 and a second expression vector comprises the polynucleotides according to SEQ ID NO: 18. Following transfection with expression vectors comprising the polynucleotides encoding the antibody light and heavy chains, as well as at least one selectable marker, transfected cells may be incubated at suitable growth conditions for 24h-72h to allow expression of the inventive

antibody, and screening for cells that have high productivity of the inventive antibody following growth recovery, serum-free suspension adaptation and amplification and clone selection.

For example, the inventive expression vector, or e.g. the at least one expression vector of the invention comprising the polynucleotides according to SEQ ID NO:17 and/or SEQ ID NO: 18 may be used to produce or manufacture the inventive antibody as disclosed above. For example, the expression vector comprising the inventive polynucleotides comprising the sequences according to SEQ ID NO:17 and/or SEQ ID NO:18 may be used to transfect at least one suitable host cell for the expression of the inventive antibody as disclosed above. For example, the inventive polynucleotides according to SEQ ID NO:17 and SEQ ID NO:18 may be comprised in one expression vector, or e.g. may be comprised in two separate expression vectors that together are used in the production of the inventive antibody.

Polynucleotides comprising the nucleotide sequences according to SEQ ID NO: 17 and SEQ ID NO:18 may e.g. be used in retroviral, or lentiviral vectors to transduce at least one suitable host cell for the expression of the inventive polynucleotides.

For example, for the manufacture or production of the inventive antibody the polynucleotide encoding the light chain amino acid sequence according to SEQ ID NO:15 and the polynucleotide encoding the heavy chain amino acid sequence according to SEQ ID NO:16 may be comprised on two separate expression vectors, such that both of the expression vectors have to be transfected into a suitable host cell for the production or manufacture of the inventive antibody. The transfection method used may e.g. depend on the host cell line utilized. A variety of transfection methods have been developed to stably introduce vector DNA into mammalian cells, including e.g. calcium phosphate, electroporation, cationic lipid-based lipofection, and polymer or dendrimer-based methods. In one example, the inventive expression vector, or the at least one expression vector according to the invention may be used in recombinase-mediated cassette exchange (RMCE) using CHOK1SV cells to generate stable cell lines for the manufacture of the inventive antibody, e.g. according to the method described in Biotechnol Prog. 2015 Nov-Dec;31(6):1645-56.

According to one embodiment the present invention provides a host cell which comprises at least one expression vector as disclosed above. For example, the host cell according to the invention may comprise at least one expression vector as disclosed above which encodes the inventive antibody comprising the amino acid sequences according to SEQ ID NO: 17 and SEQ ID NO:18, e.g. the host cell may comprise one expression vector as disclosed above encoding the amino acid sequence according to SEQ ID NO: 17 and a second expression vector as disclosed above which encodes the amino acid sequence according to SEQ ID NO: 18. The expression vectors may comprise selectable markers, e.g. antibiotic resistance genes, such as e.g. G418, zeocin, blasticidin, hygromycin B, mycophenolic acid, or puromycin. The

inventive antibody may e.g. also be encoded by more than one expression vector of the invention, each of which may comprise a different selectable marker to allow the selection of host cells which have been transfected with at least one of each of the expression vectors. For example, a first expression vector of the invention comprising the polynucleotide according to SEQ ID NO: 17 may comprise a neoR gene and a second inventive expression vector may comprise a bsd gene which confers resistance against blasticidin. Accordingly, a host cell which comprises both expression vectors of the invention will confer resistance to G418 and blasticidine and may be selected in growth medium containing both antibiotics. In one aspect the host cell according to the invention may comprise the inventive polynucleotides as disclosed above stably integrated into the host cell genome, e.g. by viral transduction of the polynucleotides according to SEQ ID NO:15 and SEQ ID NO:16 including at least one, two or more selectable markers. For example, in one aspect the host cell according to the invention may comprise the inventive polynucleotides according to SEQ ID NO: 15 and SEQ ID NO:16 as episomes, e.g. with at least one, two different selectable markers.

For example, the host cell according to the invention may be a bacterial cell, insect cell, yeast cell or mammalian cell. For example, a host cell according to the invention comprising at least one inventive polynucleotide or an expression vector according to the invention as disclosed above refers to any type of cell that can contain the vector according to the invention. The host cell can be a eukaryotic cell, e.g., plant, animal, fungi, or algae (e.g. *Phaeodactylum tricornutum*, *Chlamydomonas reinhardtii*) or can be a prokaryotic cell, e.g., bacteria or protozoa. The host cell can be a cultured cell or a primary cell, i.e., isolated directly from an organism, e.g., a human. The host cell may e.g. be an adherent cell or a suspended cell, i.e., a cell that grows in suspension. A host cell according to the invention may e.g. include *Saccharomyces cerevisiae*, *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Schwanniomyces occidentalis*, *Kluyveromyces fragilis*, *Yarrowia lipolytica* and *Pichia pastoris*, or e.g. Sf9, Sf21, S2, Hi5, or BTI-TN-5B1-4 cells, or e.g. DH5 $\alpha$  E.coli. Preferably, the host cell according to the invention is one of HEK293, HEK293T, HEK293E, HEK 293F, NS0, per.C6, MCF-7, HeLa, Cos-1, Cos-7, PC-12, 3T3, Vero, vero-76, PC3, U87, SAOS-2, LNCAP, DU145, A431, A549, B35, H1299, HUVEC, Jurkat, MDA-MB-231, MDA-MB-468, MDA-MB-435, Caco-2, CHO, CHO-K1, CHO-B11, CHO-DG44, BHK, AGE1.HN, Namalwa, WI-38, MRC-5, HepG2, L-929, RAB-9, SIRC, RK13, 11B11, 1D3, 2.4G2, A-10, B-35, C-6, F4/80, IEC-18, L2, MH1C1, NRK, NRK-49F, NRK-52E, RMC, CV-1, BT, MDBK, CPAE, MDCK.1, or MDCK.2, D-17.

According to one embodiment the present invention provides for the use of a host cell of the invention as disclosed above in the manufacture of the inventive antibody, e.g. the inventive antibody comprising the amino acid sequences according to SEQ ID NO: 15 (light chain) and SEQ ID NO:16 (heavy chain). For example, the use of a host cell according to invention in the manufacture of the inventive antibody may comprise culturing at least one host cell comprising

at least one expression vector or polynucleotide of the invention comprising the polynucleotide sequences according to SEQ ID NO: 15 and/or SEQ ID NO:16 under suitable conditions that allow the expression of the inventive antibody. In one example, the at least one host cell of the invention comprises the polynucleotides according to SEQ ID NO: 17 and SEQ ID NO: 18 including regulatory sequences required for the expression of the polynucleotides, stably integrated into the host cell's genome.

For example, the at least one host cell of the invention, e.g. at least  $10^2$ ,  $10^3$ ,  $10^4$ ,  $10^5$ ,  $10^6$ ,  $10^7$ ,  $10^8$ ,  $10^9$  cells, may be allowed to grow in DMEM containing 10% FBS at 37 °C in a 10% CO<sub>2</sub> atmosphere or e.g. in serum-free culture medium to aid in the subsequent isolation and purification such as e.g. FreeStyle™ 293 expression medium, or OptiCHO™ medium. For example, Grace's insect medium, express Five ® SFM (Life Technologies), or High Five ® medium (Life Technologies) may be used if the at least one host cells of the invention is an insect cell as disclosed above, or YNM medium, YPD broth, or e.g. PichiaPink (Life technologies) if the at least one host cells of the invention is a yeast cell. For example, suitable growth conditions may comprise allowing the at least one host cell of the invention to grow between 12-408h, e.g. for about 12 to about 400h, e.g. between 14h, 16h, 18h, 20h, 24h, 36h, 48h, 72h, 96h to about 120h, 144h, 168h, 192, 216h, 240h, 264h, 288h, 312h, 336h, 360h, 384h, 408h. Subsequently, the inventive antibody or antigen binding fragment as disclosed above may be isolated and purified. For example, the antibody or antigen-binding fragment of the invention may be purified and isolated by chromatography, e.g. ion-exchange chromatography, size-exclusion chromatography, ammonium sulfate precipitation, ultrafiltration, or purified using protein A sepharose, e.g. as described in Current Protocols in Molecular Biology (1997) 11.11.1-11.11.5.

## EXAMPLES

The following Examples are intended to further illustrate the invention. They are not intended to limit the subject matter or scope of the invention thereto.

5

### Example 1 - Antibody generation

The inventive monoclonal rabbit antibody directed against a KLH-coupled carboxyterminal peptide of PD-L1 (SEQ ID NO: 1, RLRKGRMMDVKKCGIQDTNSKKQSDTHLEET) was generated using the same basic principal for making monoclonal antibodies as for mouse monoclonal antibodies. In brief, the generation of the inventive rabbit monoclonal antibody followed a four-step procedure:

- 1) Immunization of rabbits and screening of polyclonal sera,
- 2) Fusion to generate hybridoma cells and screening of supernatants of multiclones.
- 3) C. Subcloning and screening of supernatants of subclones.
- 15 4) Cloning and production of recombinant antibody in HEK-293 cells.

The supernatants of multiclones, subclones and recombinant antibodies were screened for binding to a PD-L1 antigen having the amino acid sequence according to SEQ ID NO: 1 by enzyme-linked immunosorbent assay (Figure 1). The initial screen resulted in the identification of one antibody clone MKP1A07310 which was then further characterized by means of Western blotting and immunohistochemistry on FFPE tissue sections.

### Example 2: Characterization of antibodies by Western blotting

Antibody clone MKP1A07310 was analyzed by Western blotting on lysates of cell lines A549, MDA-MB 231, PD-L1 knockdown MDA-MB 231, and human PD-L1 transfected HEK293 (=HEK293\_PDL1) cell lines to assess its specificity. A549 and MDA-MB 231 cell lines were retrieved from the cell bank at Merck KGaA, Darmstadt. The knockdown of human PD-L1 in MDA-MB 231 cells was performed at Merck, Darmstadt using siRNA against PD-L1 (siGENOME SMARTpool, Dharmacon). The polyclonal antibody anti-CD274 (Abcam, catalogue no. 58810) was used as positive control antibody for the detection of PD-L1 (=CD274). Primary rabbit antibodies were detected with Alexa Fluor conjugated goat anti-rabbit antibody (Invitrogen, catalog no. 21076). Western Blots were scanned using Odyssey scanner (LI-COR, USA).

Western blotting was performed on cell lysates of PD-L1 transfected HEK293, PD-L1 expressing MDA-MB 231, siRNA PDL1 knockdowns of MDA-MB 231 and the PD-L1 low cell line A549. The Western Blots showed a specific labeling of PD-L1, that was reduced in the siRNA PD-L1 knockdown MDA-MB 231 (see Figure 2). PD-L1 protein has a MW of approximately 40kDa. Due to a high degree of glycosylation the MW of glycosylated PD-L1 is ~52kDa. The polyclonal positive control antibody (anti-CD274, diluted 1:250) showed several bands in addition to the specific 52 kDa band indicating that this antibody has a low selectivity for PD-L1. In comparison to the polyclonal antibody, the monoclonal recombinant antibody is highly selective.

### Example 3: Antibody cloning

mRNA from hybridoma cells of the inventive clone MKP1A07310 was isolated using TurboCapture Kit (Qiagen: Catalog #72232) following the manufacturer's recommendations and reverse transcribed into cDNA using oligo-dT primer. The variable region of heavy chain (VH) was PCR amplified using proprietary Epitomics primers OYZ64-2 and OYZvh3. The entire light chain (LC) was PCR amplified using proprietary primers OYZ62 and OYZ71. The VH region of PCR fragments was digested using restriction enzyme HindIII and KpnI. The LC PCR fragments were digested using HindIII and NotI. Following the restriction digest, the resulting fragments were purified using a Qiagen PCR cleaning up kit (catalog #28016) and the resulting purified VH and LC fragments were ligated into the corresponding heavy or light chain a proprietary pTT expression vector and transformed into competent E. coli DH5 $\alpha$  cells (MC Lab, catalog #DA-100). Resulting bacterial colonies were picked and inserts were confirmed (by expected size: approximately 440 bp for VH and 740 bp for LC) using the corresponding restriction enzymes. Plasmids with inserts of the expected size were sequenced using TT5-specific sequencing primers. Following sequence verification the entire light chain and heavy chain fragments were excised from the corresponding vector with HindIII and NotI and subsequently purified using Qiagen PCR cleaning up kit.

### Example 4: Assessment of antibody specificity

In order to establish that the inventive antibody MKP1A07310 as well as antibody MKP1B19610 specifically bind to human PD-L1, siRNA experiments using MDA-MB 231 cells were done. For MDA-MB 231 cells it has been shown that this cell line possesses a high baseline PD-L1 expression (see e.g. Cancer Immunol Res. 2014 April ; 2(4): 361–370). Immunohistochemical staining of siRNA transfected MDA-MB 231 cells showed that both antibodies tested specifically recognized and bound to PD-L1 (see: Figure 3): Upon

transfection of siRNA1, or siRNA2 the antibody staining for PD-L1 was strongly reduced (see: Figure 3, "PDL1\_SP+", "PDL1\_SP"). This initial determination of the binding specificities of both antibodies already indicated that MKP1B19610 appeared to be less sensitive, since greater antibody concentrations were required (5µg/ml or greater) to result in comparable staining intensities as observed for the inventive antibody MKP1A07310 (e.g. at a concentration of 0.2µg/ml).

For the assessment of the specificity of the inventive antibody clone MKP1A07310 by means of immunohistochemistry a tissue microarray (TMA) generated out of positive and negative control tissue with known PD-L1 protein expression was used. For the construction of a tissue microarray, human normal and tumor tissue with different PD-L1 expression levels were selected. The expression of PD-L1 was analyzed previously on a frozen tissue microarray of human organs (BioChip, Indivumed GmbH) and on frozen human tumors using the inventive anti-PD-L1 antibody. Paraffin blocks of PD-L1 positive and negative normal human tissues were purchased from provitro GmbH (Germany). From selected patients with high or low PD-L1 expression, the corresponding paraffin blocks of non-small-cell lung carcinoma (NSCLC) tumors were purchased from Indivumed GmbH (Germany). Out of each paraffin block two punches of 1.5 mm in diameter were taken and mounted upright on an adhesive tape and incorporated in liquid paraffin (see e.g. Figure 7).

#### Immunohistochemistry on FFPE tissue or tissue/cell line arrays

Sections of 3µm of FFPE tissue or tissue/cell microarrays were mounted on positively charged SuperFrost®Plus slides (Menzel-Gläser, Braunschweig, Germany). The immunohistochemical staining procedure, starting with the deparaffinization of sections was done with the staining instrument Discovery™, or Discovery® XT (Ventana MedicalSystems, Inc., Tucson, USA; see diagram below). After deparaffinization, sections were heated for epitope retrieval in Tris-EDTA buffer pH 8. Endogenous peroxidase was blocked by incubation in 3% hydrogen peroxide (part of OmniMap™ Kit, Ventana Medical Systems). Sections were incubated with in PBS diluted antibodies and then with the secondary antibody, the HRP conjugated polymers of the OmniMap Kit, for 16 min at 37°C. Horseradish peroxidase (HRP) catalyzes the 3,3'-diaminobenzidine tetrahydrochloride (DAB)/H<sub>2</sub>O<sub>2</sub> reaction to produce an insoluble dark brown precipitate that can be visualized. A monoclonal rabbit IgG antibody served as negative control. Sections were counterstained with hematoxylin. Slides were washed in tap water, dehydrated, and mounted with glass coverslips in permanent mounting media Entellan® Neu (VWR, Germany).

Immunohistochemical staining procedure on Discovery™ Instruments

For the immunohistochemical staining of the FFPE tissues the following Discovery™ platforms (Ventana Medical Systems, INc.) were used: Catalog No. 750-200, 750-800, Catalog No F-DISXT-750-000, according to the following steps:

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**Tissue/Probe:** Human tissue, cancer cell lines in vitro,

**Preservation:** FFPE (4% paraformaldehyde pH 7, paraffin embedded)

**Section thickness:** 3 µm for FFPE tissue

**1. Baking and deparaffinization of sections**

10 Baking of slides during 8 min at 75°C

Deparaffinization of slides during 8 min at 75°C

**2. Blocking** blocking of endogene peroxidase is prior to proteinase antigen retrieval and after heat antigen retrieval in the Discovery staining instrument

Blocking of endogene peroxidase with 3% H<sub>2</sub>O<sub>2</sub> at 37°C15 **3. Pretreatment of sections for antigen retrieval**

standard CC1 (Tris EDTA buffer pH 8) during 48 min at 96°C

**4. Incubation with primary antibody**Primary Antibody: **PDL1 (inventive antibody MKP1A07310)**

Company: Merck KGaA, Darmstadt

20 Catalogue no: MKP-1A-73-10

Isotype: rabbit monoclonal recombinant

Clone: MKP1A07310

Dilution or Concentration: 1- 2 µg/ml

Incubation during 32 min at 37°C

25 Negative control for primary antibody

is: rabbit mAb IgG isotype control, clone DA1E (NEB, #3900)

**5. Incubation with secondary antibody**

Secondary Antibody: anti-rabbit IgG from OmniMap anti-rabbit HRP

Company: Ventana

30 Catalogue no: 760-4311

Dilution: VMSI Dispenser (unknown)

Incubation during 16 min at 37°C

**6. Incubation with tertiary antibody or detection kit used**

Antibody/Kit:

35 OmniMap anti-rabbit HRP (#760-4311, VMSI), ChromoMap (#760-159, VMSI)

Duration of the incubation, temperature, and washing steps of the ChromoMap (DAB, copper) is set by the Discovery instrument



**7. Counterstain** Hematoxylin II (#790-2208, VMSI) during 8 min at 37°C

Bluing by instrument

Washing steps are not included in the diagram. Reagents for deparaffinization, antigen

- 5 retrieval, washing of slides are from VMSI (Ventana Medical Systems, Inc.). Antibodies are diluted in PBS.

Image analysis

- 10 Immunohistochemical stainings were scanned with the help of the MiraxSCAN (Zeiss, Germany) with a resolution x/y: 1 pixel = 0.23 x 0.23  $\mu\text{m}^2$ . The MiraxScan instruments calibrates brightness for every slide prior to scanning. Images were taken with the help of the MiraxViewer software. The scanings were analysed with the image analysis software Visiopharm Integrator System (VIS). Viable tissue area was outlined avoiding obvious necrotic areas and connective tissue.

- 15 For the determination of the amount of antigen present, positive brown stained area was calculated as percent area of the viable tissue area. Antibody staining (arbitrary units) is calculated as

- 20 Antibody staining (AU) = Positive area (%) \* (255-Intensity)/100 of the brown color, whereby the positive area was calculated as Positive area (%) =  $100 * [\text{brown area} / (\text{brown area} + \text{blue area})]$ , and intensity was set as the mean grey value of the brown area (see Figure 7A, arrows in the most right image indicate brown cells, arrows the middle image indicate the software-based detection of the brown chromogen, right image: detection of blue staining of nuclei and cytoplasm).

25

**Example 5:** Intra- and Inter-run variability of MKP1A07310 and MKP1B19610

- 30 The inventive antibody MKP1A07310 and the antibody MKP1B19610 were characterized in two concentrations each on a cancer cell line array out of 70 cell lines to compare their binding characteristics and determine the optimum concentrations for further studies. The cancer cell line array CAX09\_70 showed a continuous range of binding of the antibodies. In ~ 50% of the cell lines the signal was zero or very low. The highest binding of the antibodies showed Hs746T cells (Met amplified cell line). The antibody MKP1A07310 showed only minor increase of signal intensity upon increasing its concentration from 1 to 2  $\mu\text{g/ml}$ , indicating that 1  $\mu\text{g/ml}$  was
- 35 already near the saturating concentration. Antibody MKP1B19610 showed substantial increase in signal from 5 to 10  $\mu\text{g/ml}$ . Higher concentrations were not tested, because antibodies tend to bind unspecifically onto tissue and glass at concentrations > 10  $\mu\text{g/ml}$ . The

binding of the antibodies MKP1A07310 and MKP1B19610 on the 70 cell lines correlate with a Pearson coefficient of  $r = 0.95$ .

Intra-run and inter-run variability of immunohistochemical staining using the two antibodies on the Discovery XT staining instrument were performed on a cell line array out of 60 cell lines, CAX08\_60 (Figure 6). Antibody MKP1A07310 was tested at a concentration of 1  $\mu\text{g/ml}$  and antibody MKP1B19610 at 10  $\mu\text{g/ml}$ . On the cell lines of the CAX08\_60 the staining of MKP1A07310 correlated with MKP1B19610 with a Pearson coefficient of  $r = 0.97$ . In cell lines showing a medium to low overall staining signal, like A549, PC-3 or U-87 MG, cells showing high staining were dispersed between negative cells (Figure 9A: MKP1A07310; Figure 9B: MKP1B19610).

Intra-run variability of the inventive antibody MKP1A07310 was low, with correlation coefficients of  $r = 0.99$  between different slides (Figure 5). MKP1B19610 showed a slightly higher intra-run variability, with correlation coefficients ranging from 0.93 to 0.99 between different slides. Inter-run variability between 3 different runs was low with  $r = 0.99$  for MKP1A07310 and  $r \geq 0.98$  for MKP1B19610.

The recombinant antibodies were additionally tested on tissue microarrays comprised out of 48 different xenografts (TMA\_Xenos\_12, Figure 8). Staining with MKP1A07310 correlated with MKP1B19610 with a correlation coefficient of  $r = 0.90$ . High expressing xenografts were Hs746T, MDA-MB 231, NCI-H2009, and H1975. Negative examples were A2780 and Colo205.

#### **Example 6:** Comparison of MKP1A07310 and MKP1B19610 using different IHC platforms

Aim of the study was to compare the binding characteristics of the antibodies using the AutostainerLink 48 together with the PT Link modules for antigen retrieval from Dako (Dako GmbH, Germany) with the stains using the instrument Discovery XT. The reagents used by Dako or Ventana Medical Systems, Inc. (VMSI) for deparaffinization, antigen retrieval, dilution of antibodies on the slide, washing buffer, and detection systems differ and might influence the "epitope world" of the antibodies

#### **Substrates**

A tissue microarray out of positive and negative control tissue for PD-L1, the TMA\_PDL1\_11, and a cell line microarray out of 59 human cancer cell lines and 1 insect cell line, CAX08\_60, were used as a test matrix. The TMA\_PDL1\_11 was used for the selection of the antibodies generated. Sections of 3  $\mu\text{m}$  of formaldehyde fixed paraffin embedded (FFPE) tissue or

tissue/cell microarrays were mounted on positively charged SuperFrost®Plus slides (Menzel-Gläser, Braunschweig, Germany).

Antibodies used:

- 5 Primary antibodies: The inventive antibody MKP1A07310 ("7310 mAb") and MKP1B19610 were used diluted into PBS: MKP1A07310 was used concentrations of 1, 2 and 5 µg/ml; antibody MKP1B19610 was used at concentrations of 2, 5, and 10 µg/ml;

Secondary antibodies

Antigen	Antibody	Vendor	Conc./dilution	Incubation
Rabbit IgG	Oligomer, HRP coupled (OmniMap)	VMSI#760-4311	Dispenser	16 min @ 37°C
Rabbit IgG	Oligomer, HRP coupled (EnVision Flex/HRP)	Dako, #SM802	Dispenser	30 min @ RT

10

## Staining Procedures

### Antigen retrieval

- Antigen retrieval was done as follows: For the Discovery XT antigen retrieval was done using "High pH" antigen retrieval conditions (Tris-EDTA pH 8), for the AutostainerLink, testing started
- 15 by using "High pH" (Tris-EDTA buffer, pH 9) antigen retrieval. For antibody PD-L1 MKP1B19610) in addition "Low pH" (Citrate buffer, pH 6.1) antigen retrieval was used.

### Staining procedure for Discovery XT staining instrument

- 20 The immunohistochemical staining procedure starting with the deparaffinization of sections was done with the staining instrument Discovery® XT (Ventana Medical Systems, Inc., ("VMSI") Tucson, USA). After deparaffinization sections were heated for epitope retrieval in Tris-EDTA buffer pH 8 at 96°C for 48 min. Endogenous peroxidase was blocked by incubation in 3% hydrogen peroxide (part of OmniMap™ Kit, Ventana Medical Systems). Sections were
- 25 incubated with in PBS diluted antibodies and then with the secondary antibody, the HRP conjugated polymers of the OmniMap Kit (Discovery) for 16 min at 37°C. Horseradish

peroxidase (HRP) catalyzes the 3,3'-diaminobenzidinetetrahydrochloride (DAB)/H<sub>2</sub>O<sub>2</sub> reaction to produce an insoluble dark brown precipitate that can be visualized. Sections were counterstained with hematoxylin. The inventive anti-PD-L1 antibody was used in concentrations of 1, 2 and 5 µg/ml; antibody MKP1B19610 was used at 2, 5, and 10 µg/ml; for both antibodies detection was done using OmniMap anti-rabbit HRP (#760-4311, VMSI) and ChromoMap (#760-159).

#### Staining procedure for AutostainerLink 48

Deparaffinization and antigen retrieval of sections was done in the PT link pretreatment module (Dako GmbH, Germany). Slides were deparaffinated during heating for epitope retrieval in Tris-EDTA buffer pH 9 (High pH) or in citrate buffer pH 6.1 (Low pH) at 97°C during 20 min. After heat antigen retrieval, slides were transferred into the AutostainerLink 48 instrument. Endogenous peroxidase was blocked by incubation in 3% hydrogen peroxide. Sections were incubated with in PBS diluted antibodies and then with the secondary antibody, the HRP conjugated polymers of the EnVision FLEX (SM802, Dako) for 30 min at room temperature. Horseradish peroxidase (HRP) catalyzes the 3,3'- diaminobenzidine tetrahydrochloride (DAB)/H<sub>2</sub>O<sub>2</sub> reaction to produce an insoluble dark brown precipitate that can be visualized. Sections were counterstained with hematoxylin. Slides were subsequently washed in tap water, dehydrated, and mounted with glass coverslips in permanent mounting media Entellan® Neu (VWR, Germany). MKP1A07310 was used at concentration of 0.5, 1 and 2 µg/ml, MKP1B19610 at concentrations of 0.2, 0.5, 1, 2, and 5 µg/ml.

#### Comparison of MKP1A07310 and MKP1B19610 on different staining platforms

Using the AutostainerLink, the correlation of IHC results obtained with the anti- PD-L1 antibody MKP1B19610 and the inventive anti- PD-L1 antibody MKP1A07310 was low with  $r < 0.71$  (Figures 11 and 14). There was still a correlation of staining intensities in certain cell lines, as for example NCI-H2009 and Lox (see: Figure 11), but for A2780 cells that serve as negative control for PD-L1 expression, anti-PD-L1 antibody MKP1B19610 indicates PD-L1 expression, whereas the same cell line is negative for PD-L1 expression using the inventive antibody (see: Figure 11).

A comparison of the staining characteristics of the inventive anti-PD-L1 antibody (MKP1A07310) on the Discovery XT system with its characteristics using the AutostainerLink, revealed a high correlation of the IHC staining intensities on the cancer cell lines used with a correlation coefficient of  $r = 0.96$  at 0.5 µg/ml (Figure 13). Based on the present data, the reagents that were used according to the guidelines by Dako for use with the AutostainerLink

for deparaffinization, antigen retrieval and staining did not alter the IHC staining pattern by e.g. unmasking aberrant binding sites for the inventive anti- PD-L1 antibody (MKP1A07310) when compared to the outcome of the IHC staining using Ventana's Discovery XT platform and Ventana reagents, except for increased IHC staining signals.

5

Comparing the staining characteristics of the anti-PD-L1 antibody MKP1B19610 which is directed against an extracellular epitope of human PD-L1 on the Discovery XT System with its characteristics using the AutostainerLink (Dako), revealed a far lower correlation of the IHC staining intensities in the cancer cell lines used with a correlation coefficient of  $r < 0.67$  (Figure 12). Using "Low pH" antigen retrieval, the correlation was even lower than for the IHC staining conditions "High pH" (see e.g. Figure 12 B, "low pH",  $r=0.43$  at an antibody concentration of MKP1B19610 of  $5\mu\text{g/ml}$  and  $r=0.66$  at an antibody concentration of MKP1B19610 of  $1\mu\text{g/ml}$ .) There was still a correlation of IHC staining intensities in many cell lines assessed, but a group of cells, that are negative on the Discovery XT System assayed positive for PD-L1 expression using the AutostainerLink as is shown for A2780 (see e.g. Figure 11).

10

15

In conclusion, using the AutostainerLink platform with reagents from Dako, the inventive antibody directed against the cytoplasmic domain of PD-L1 (MKP1A07310) did show the same binding characteristics in 55 FFPE cancer cell lines as on the Discovery XT staining platform from Ventana Medical Systems. In contrast, additional binding sites of the antibody MKP1B19610, generated against the extracellular domain, were unmasked using the AutostainerLink instead of the Discovery XT. Thus, the inventive antibody can surprisingly be used on both widely used commercial IHC platforms, the AutostainerLink platform (DAKO) as well as on the Discovery XT staining platform from Ventana Medical Systems.

20

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In addition, as shown in Figure 5, the inter-run variability of the staining results obtained using the inventive antibody is surprisingly low as indicated by a correlation efficient of  $r=0.99$  such that the IHC staining and e.g. the derived scoring of the PD-L1 expression in a FFPE tumor tissue as disclosed herein, will be comparable between different staining runs. The inventive antibody thus meets the unmet need of an anti-PD-L1 antibody which is able to reliably detected PD-L1 expression in FFPE tumor tissue samples (e.g. derived from biopsies) that are routinely used in pathology on two commonly used IHC platforms (e.g. Discovery XT® System, Ventana and AutostainerLink, Dako).

30

## CLAIMS

1. An antibody or antigen-binding fragment thereof that binds to an epitope comprised in the amino acid sequence according to SEQ ID NO:1, wherein the antibody light chain or antigen-binding fragment thereof comprises at least three amino acid sequences according of SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 and the antibody heavy chain or antigen binding fragment thereof comprise at least three amino acid sequences of SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14.
2. Antigen-binding fragment according to claim 1, wherein the antigen-binding fragment is a Fab.
3. Antigen-binding fragment according to claim 1, wherein the antigen-binding fragment is a F(ab')<sub>2</sub>,
4. Antigen-binding fragment according to claim 1, wherein the antigen-binding fragment is a Fab'.
5. Antigen-binding fragment according to claim 1, wherein the antigen-binding fragment is a scFv.
6. Antigen-binding fragment according to claim 1, wherein the antigen-binding fragment is a di-scFv,
7. Antibody according to claim 1, wherein the antibody is a monoclonal antibody.
8. Antibody according to claim 7, wherein the monoclonal antibody is an IgG type monoclonal antibody.
9. Antibody or antigen-binding fragment thereof according to any one of claim 2-8, wherein the antibody light chain or antigen binding fragment thereof comprises all of SEQ ID NO:3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8.
10. Antibody or antigen-binding fragment thereof according to any one of claim 2-9, wherein the antibody heavy chain or antigen binding fragment thereof comprises all of SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14.
11. Antibody or antigen-binding fragment thereof according to any one of claims 1-8, wherein the antibody heavy chain or antigen-binding fragment thereof comprises the amino acid sequence according to SEQ ID NO: 110 and the antibody light chain or

antigen-binding fragment thereof comprises the amino acid sequence according to SEQ ID NO: 111.

12. Antibody according to claim 11, wherein the antibody light chain comprises the amino acid sequence according to SEQ ID NO: 15 and the antibody heavy chain comprises the amino acid sequence according to SEQ ID NO: 16.

13. Antibody according to claim 11, wherein the antibody light chain comprises the amino acid sequence according to SEQ ID NO: 114 and the antibody heavy chain comprises the amino acid sequence according to SEQ ID NO: 115.

14. Antibody according to claim 13, wherein the antibody is a rabbit antibody.

15. Antibody according to claim 13, wherein the antibody is a rabbit-derived antibody.

16. Antibody or antigen-binding fragment thereof according to any one of claims 1-15, wherein said antibody or antigen-binding fragment thereof is further coupled to a detectable label.

17. Antibody or antigen-binding fragment thereof according to claim 16, wherein the detectable label is an enzyme.

18. Antibody or antigen-binding fragment thereof according to claim 16, wherein the detectable label is a fluorophore.

19. Antibody or antigen-binding fragment thereof according to claim 16, wherein the detectable label is a radioisotope.

20. Antibody or antigen binding fragment thereof according to any one of claims 1-19 for use in detecting the presence or expression of an epitope comprised in SEQ ID NO: 1 in a sample.

21. Antibody or antigen binding fragment thereof for use according to claim 20, wherein the sample is a biological sample.

22. Antibody or antigen binding fragment thereof for use according to claim 21, wherein the biological sample is a tissue sample.

23. Antibody or antigen binding fragment thereof for use according to claim 21, wherein the sample is a fixed tissue sample.

24. Antibody or antigen binding fragment thereof for use according to claim 21, wherein the sample is a formaldehyde-fixed paraffin-embedded (FFPE) tissue.

25. Antibody or antigen binding fragment thereof for use according to any one of claims 20-24, wherein the tissue sample is derived from tumor tissue.

26. Antibody or antigen binding fragment thereof for use according to any one of claims 20-25, wherein the detection is done by means of flow cytometry.
27. Antibody or antigen binding fragment thereof for use according to any one of claims 20-25, wherein the detection is done by means of an ELISA.
- 5 28. Antibody or antigen binding fragment thereof for use according to any one of claims 20-25, wherein the detection is done by means of Western blotting.
29. Antibody or antigen binding fragment thereof for use according to any one of claims 20-25, wherein the detection is done by means of immunohistochemistry (IHC).
- 10 30. Method of detecting the presence or expression of human PD-L1 or any fragment thereof comprising the amino acid sequence according to SEQ ID NO: 1 in a sample, wherein the method comprises the step of contacting said sample with an antibody or antigen binding fragment thereof according to any one of claims 1-19, and detecting the presence of bound antibody or antigen binding fragment thereof.
31. Method according to claim 30, wherein the sample is a biological sample.
- 15 32. Method according to claim 31, wherein the biological sample is a tissue sample.
33. Method according to claim 31, wherein the biological sample is a fixed tissue sample.
34. Method according to claim 33, wherein the fixed tissue sample is a formaldehyde-fixed paraffin-embedded (FFPE) tissue sample.
- 20 35. Method according to claim 33, wherein the fixed tissue sample is a formaldehyde-fixed paraffin-embedded (FFPE) tumor tissue sample.
36. Method according to any one of claims 30-35, wherein the sample is derived from a subject having, or at risk of cancer.
37. Method according to any one of claims 30-35, wherein the sample is derived from a subject having T-cell dysfunction.
- 25 38. Method according to any one of claims 30-35, wherein the sample is derived from a subject having an acute or a chronic infection.
39. Method according to any one of claims 30-35, wherein the sample is derived from a subject being at risk of, or showing tumor immunity.
- 30 40. Use of an antibody or antigen-binding fragment thereof according to one of claims 1-18 in a method according to any one of claims 30-35.



41. Isolated polynucleotide encoding the antibody or antigen binding fragment thereof according to any one of claims 1-15.

42. Expression vector comprising the polynucleotide according to claim 41.

43. Use of an expression vector according to claim 42 in the manufacture of an antibody according to any one of claims 11-15, wherein the antibody comprises the amino acid sequences according to SEQ ID NO: 15 and SEQ ID NO:16.

44. Host cell comprising at least one expression vector according to claim 41.

45. Use of a host cell according to claim 43 in the manufacture the antibody according to claim 12.

46. Method of treating cancer in a patient comprising the steps of

- (i) detecting the presence or expression of human PD-L1 according to any one of claims 30-35 in a sample, and
- (ii) comparing the results of said PD-L1 expression from (i) to a reference sample, and
- (iii) administering an immune checkpoint inhibitor to said patient if the PD-L1 expression in the sample as detected in step (i) is increased compared to the reference sample.

47. Method according to claim 46, wherein the reference sample is derived or obtained from a healthy subject, not inflicted with cancer.

48. Method according to claim 46 or claim 47, wherein the immune checkpoint inhibitor of step (iii) which is administered to the patient is an anti-PD-L1 antibody, or anti-PD-1 antibody.

49. Method according to any of claims 46-48, wherein the immune checkpoint inhibitor is administered in a dose from about 5mg/kg body weight to about 30 mg/kg body weight.

50. Method according to any of claims 45-47, wherein the immune checkpoint inhibitor is administered in a flat dosing regimen of 500-800 mg every week, or 900-1600 mg every two weeks, or 1250 – 2400 mg every three weeks.

## DRAWINGS

Samples O.D	Coating w/MKP-1A Antigen ( 1 ug/ml@50ul/well)									
	MKP-1A-73-10									
Dilution	Unpurified Antibody		Purified Antibody		Flow Through		N/A		N/A	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1:250	1.20	1.24	1.82	2.00	0.13	0.10	0.07	0.08	0.07	0.08
1:750	0.99	0.90	1.79	1.81	0.10	0.09	0.07	0.07	0.06	0.07
1:2250	0.52	0.49	1.97	2.26	0.08	0.07	0.08	0.06	0.06	0.08
1:6750	0.23	0.22	1.96	1.89	0.07	0.06	0.07	0.06	0.07	0.07
1:20250	0.13	0.12	1.30	1.31	0.07	0.07	0.06	0.08	0.08	0.06
1:60750	0.08	0.09	0.71	0.68	0.08	0.06	0.06	0.07	0.07	0.07
1:182250	0.07	0.08	0.36	0.33	0.07	0.06	0.07	0.06	0.06	0.07
1xPBS	0.05	0.06	0.08	0.08	0.07	0.07	0.06	0.07	0.07	0.06

**ELISA Brief Protocol:**

- 1) MKP-1A antigen coated (1 ug/ml, 50 ul/well), over night
- 2) Samples at serial dilutions (50 ul/well), RT, 1.5 hr.
- 3) AP Gt x Rb IgG (1:2500), 50 ul/well, RT, 1 hr.
- 4) AP substrate, 50 ul/well, RT, 15 min
- 5) Stopping solution, 50 ul/well, OD405nm

FIGURE 1

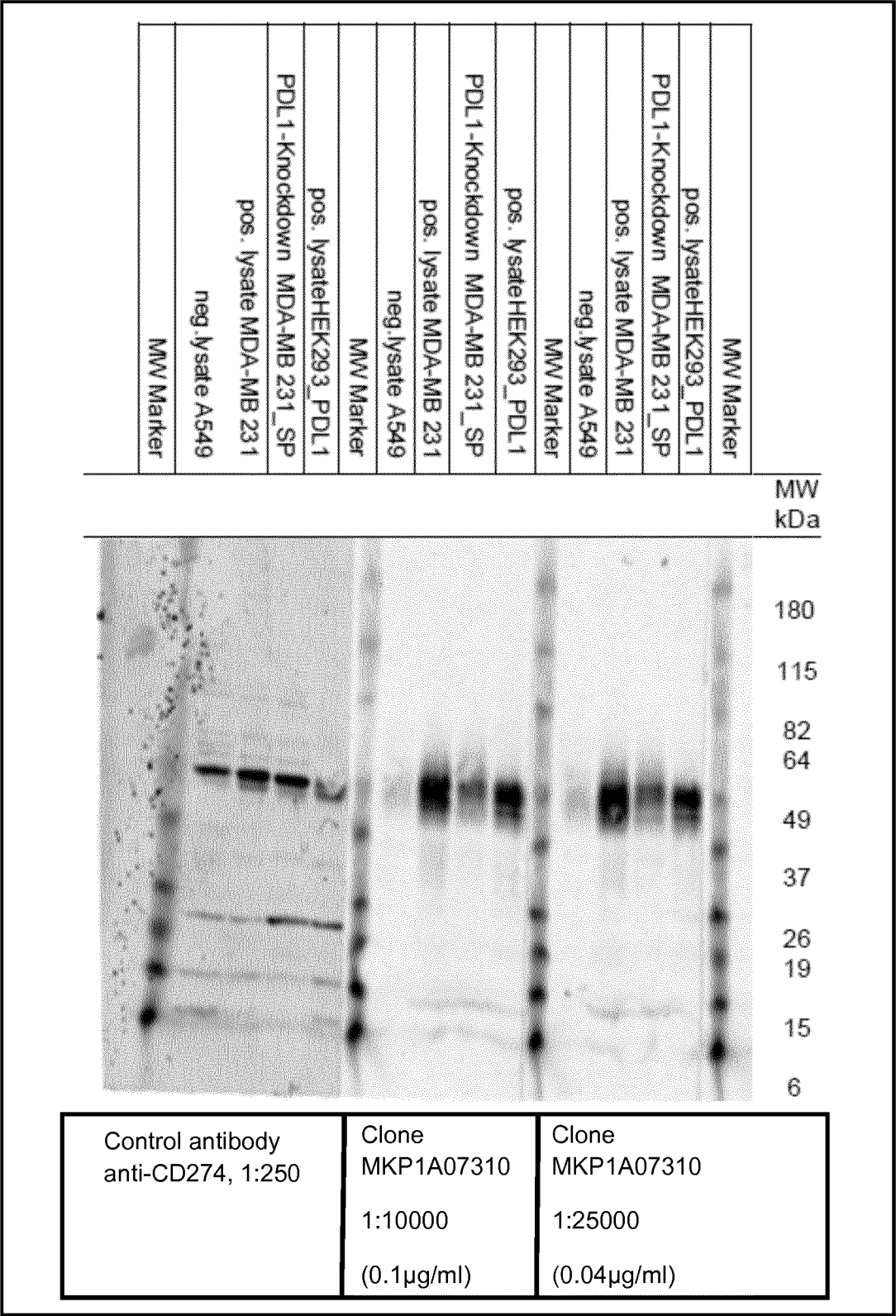
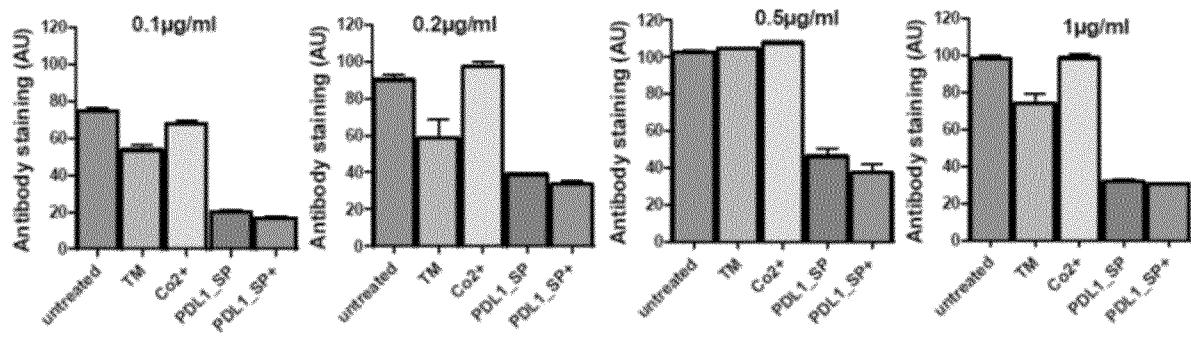
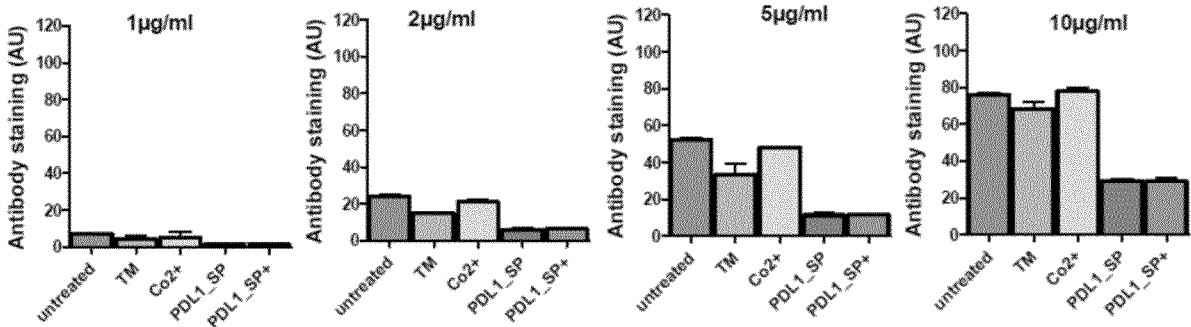


FIGURE 2

A



B



Cell line MDA-MB 231	
untreated	Untreated
TM	Treated with transfection medium
Co2+	Treated with control siRNA
PDL1_SP+	Treated with PD-L1 siRNA 2
PDL1_SP	Treated with PD-L1 siRNA 1

FIGURE 3

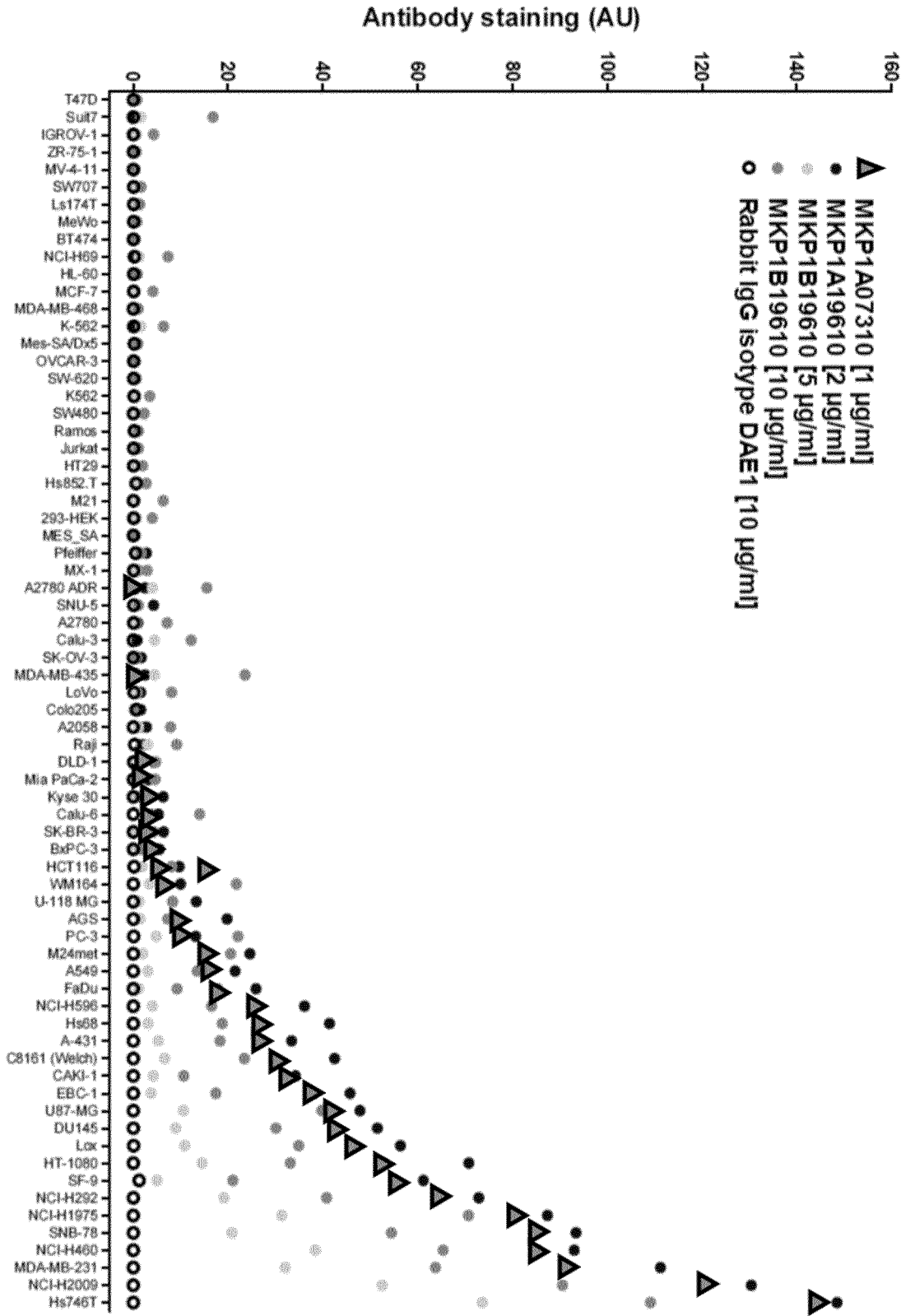


FIGURE 4

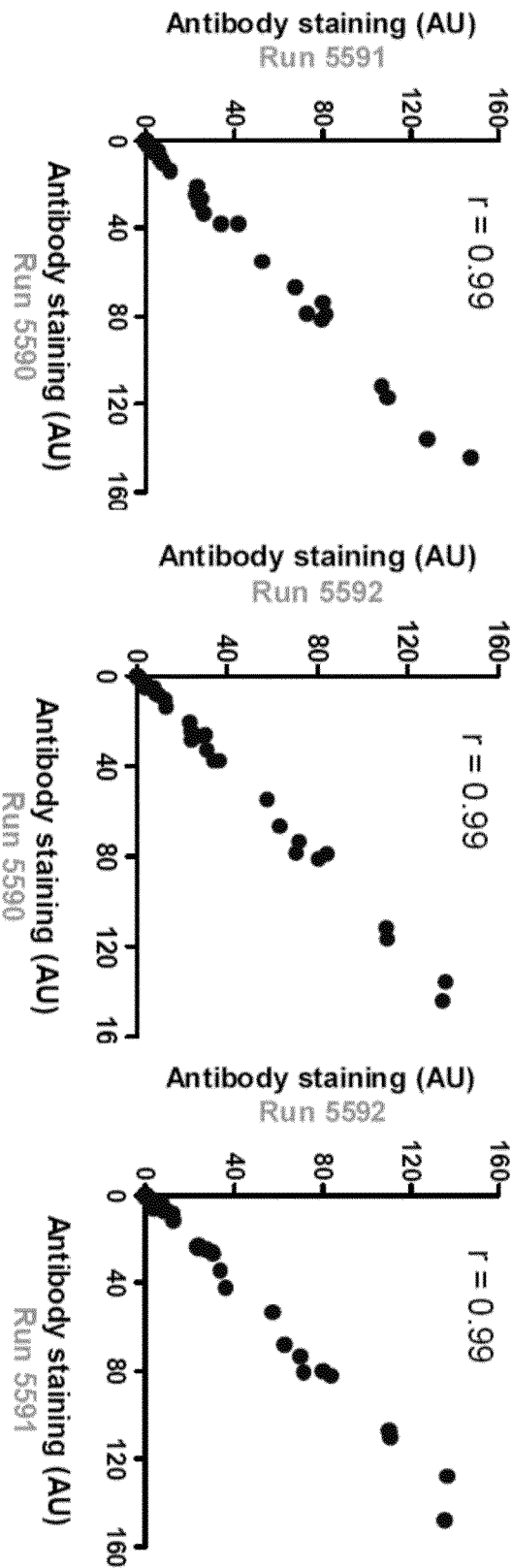


FIGURE 5

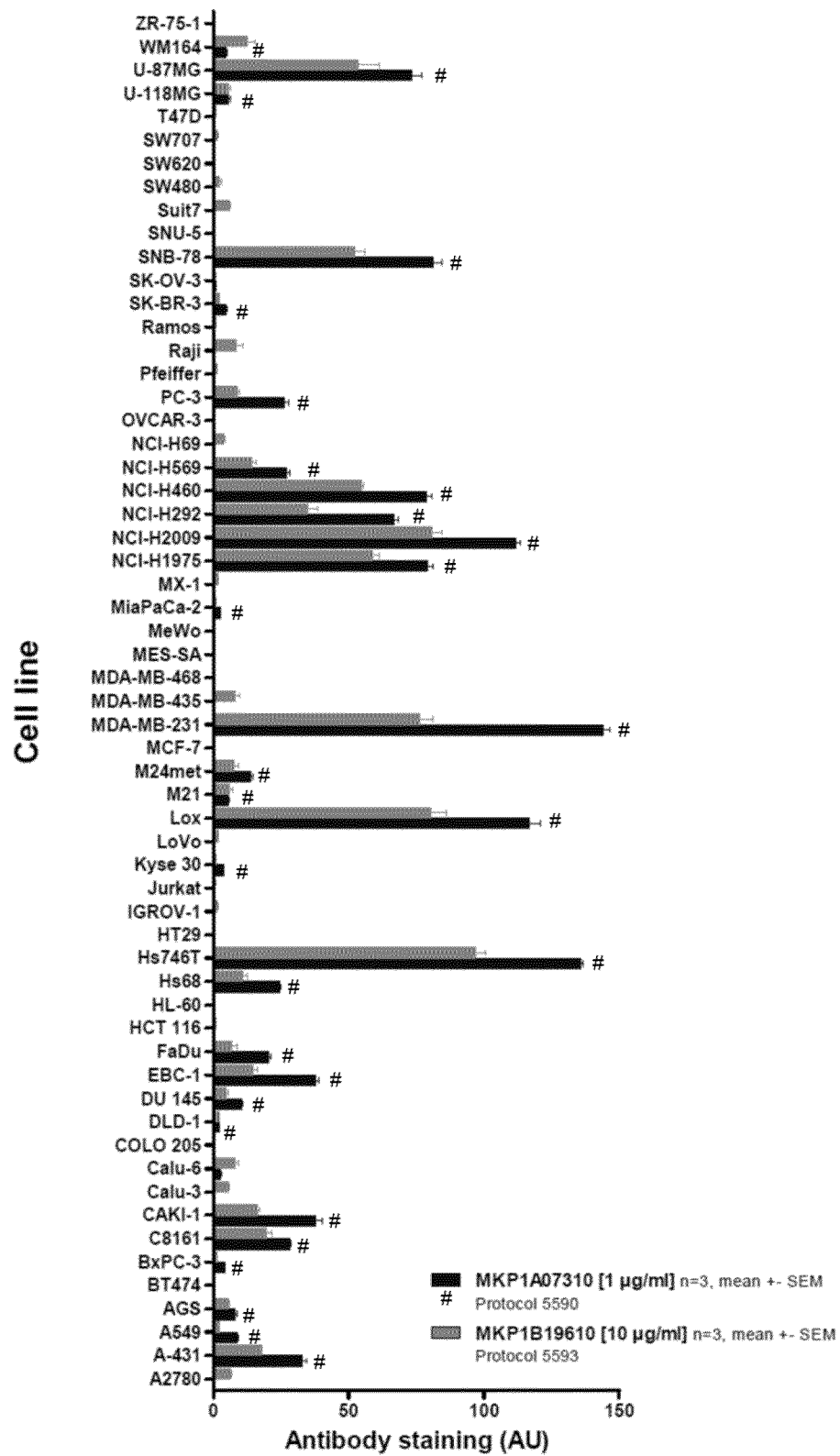
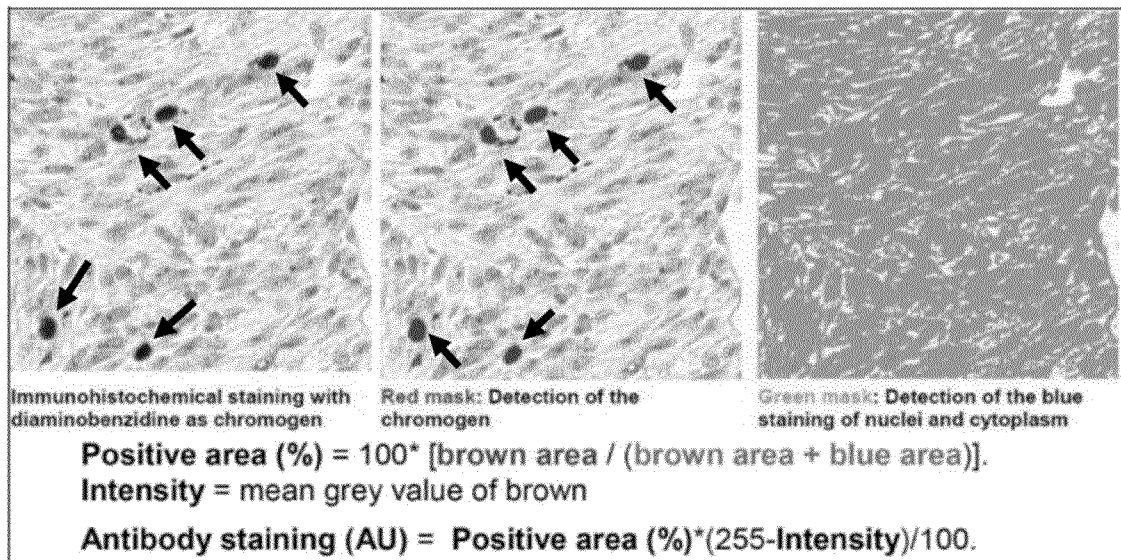


FIGURE 6

A



B

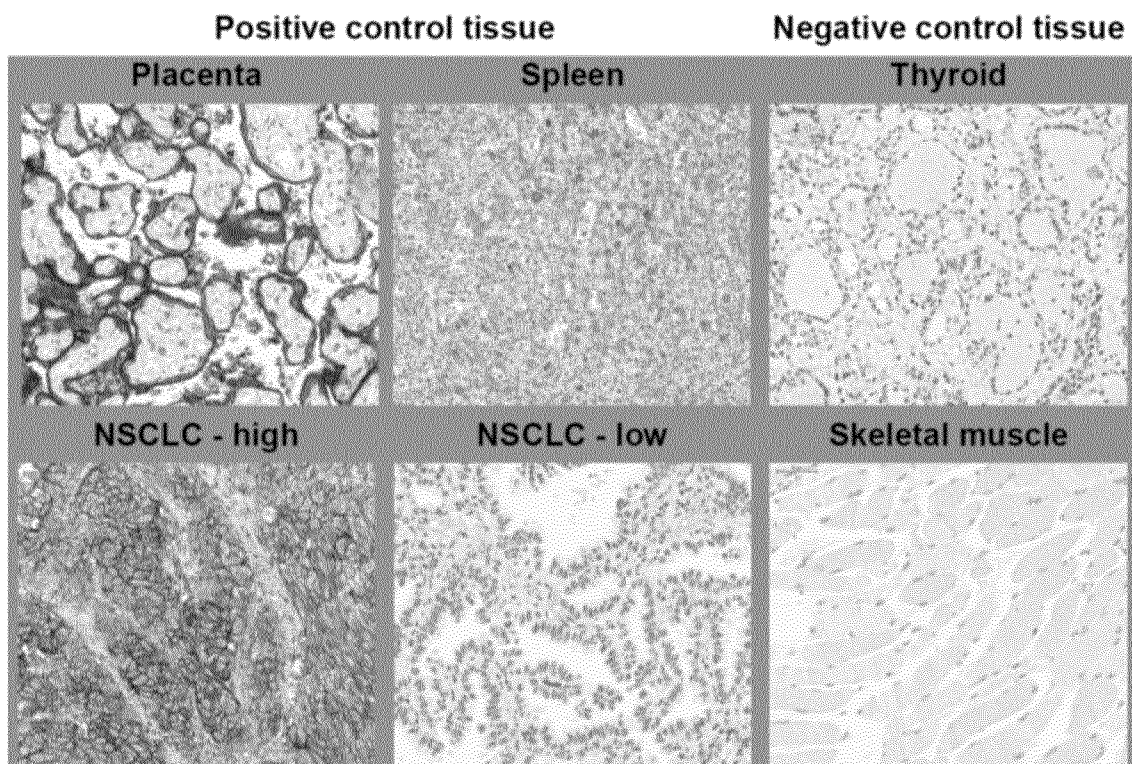


FIGURE 7



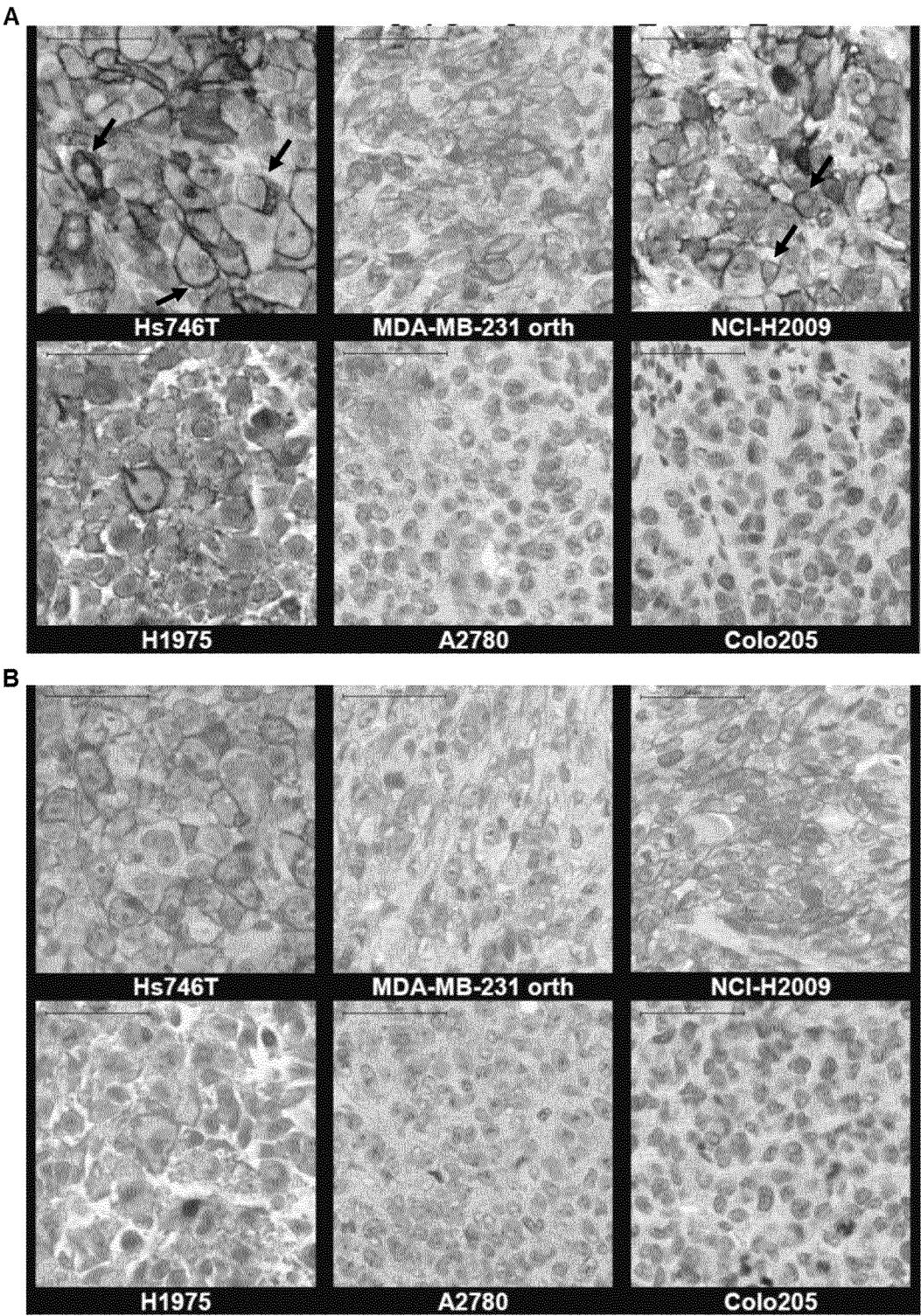
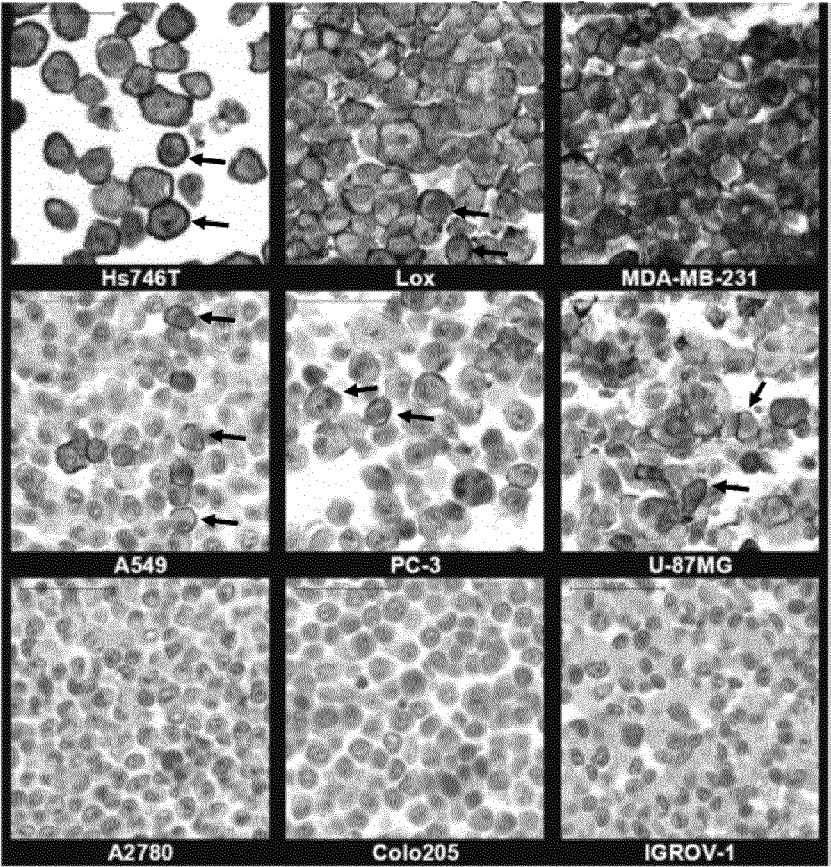


FIGURE 8

A



B

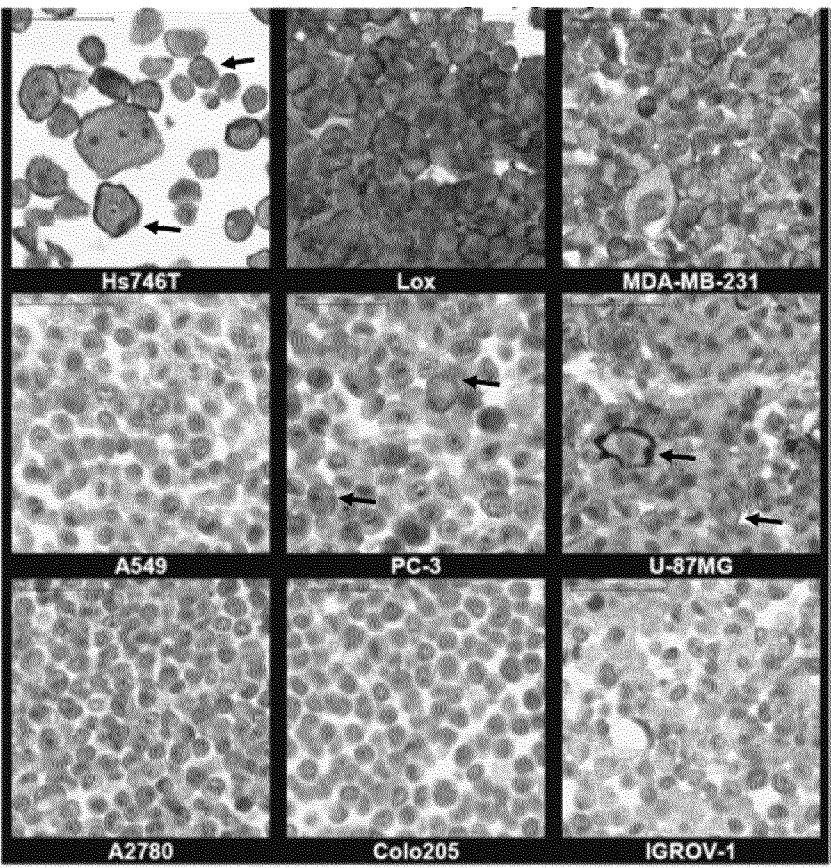


FIGURE 9

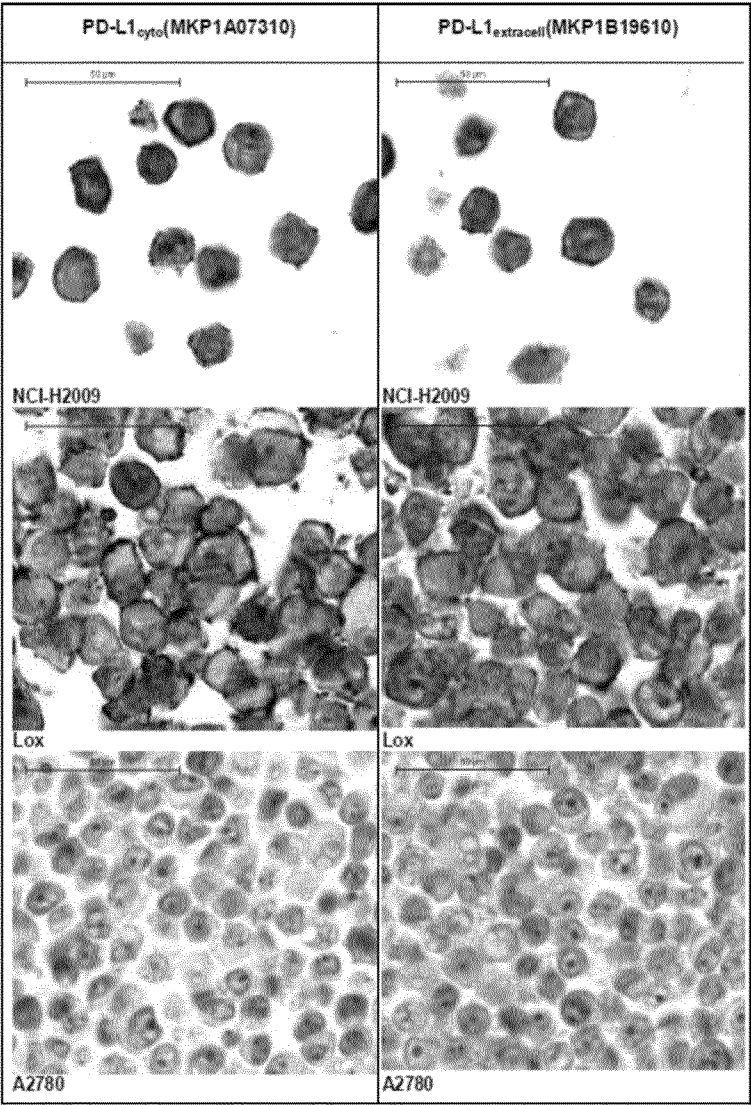


FIGURE 10

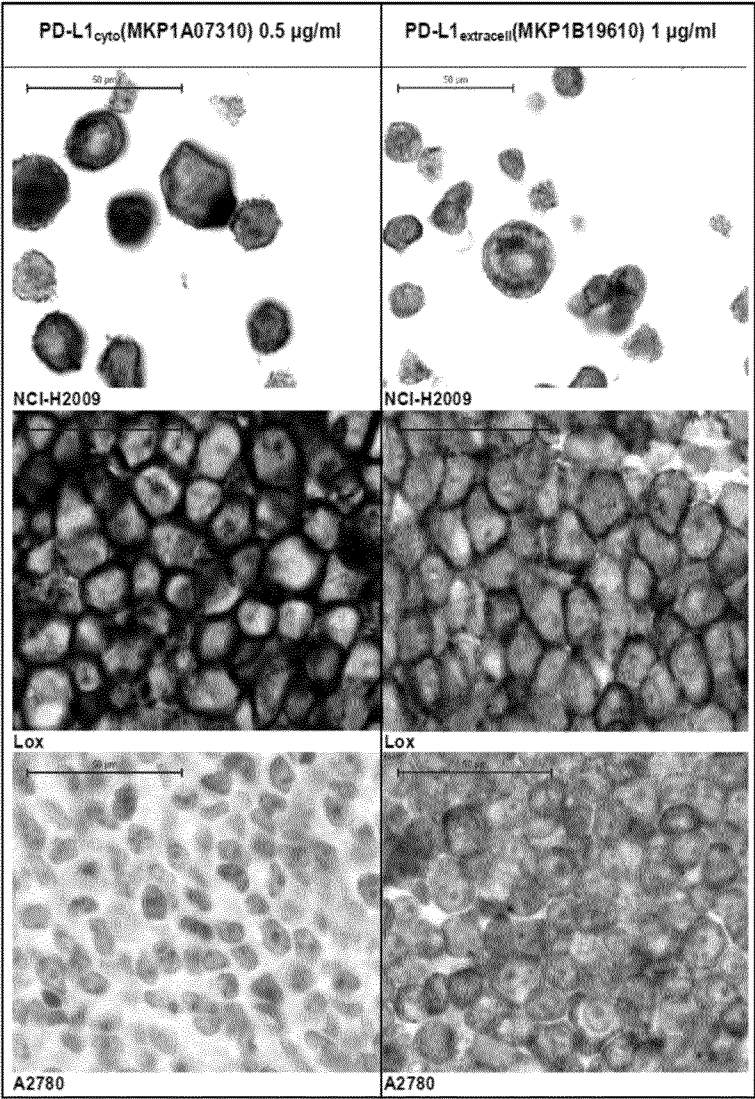


FIGURE 11

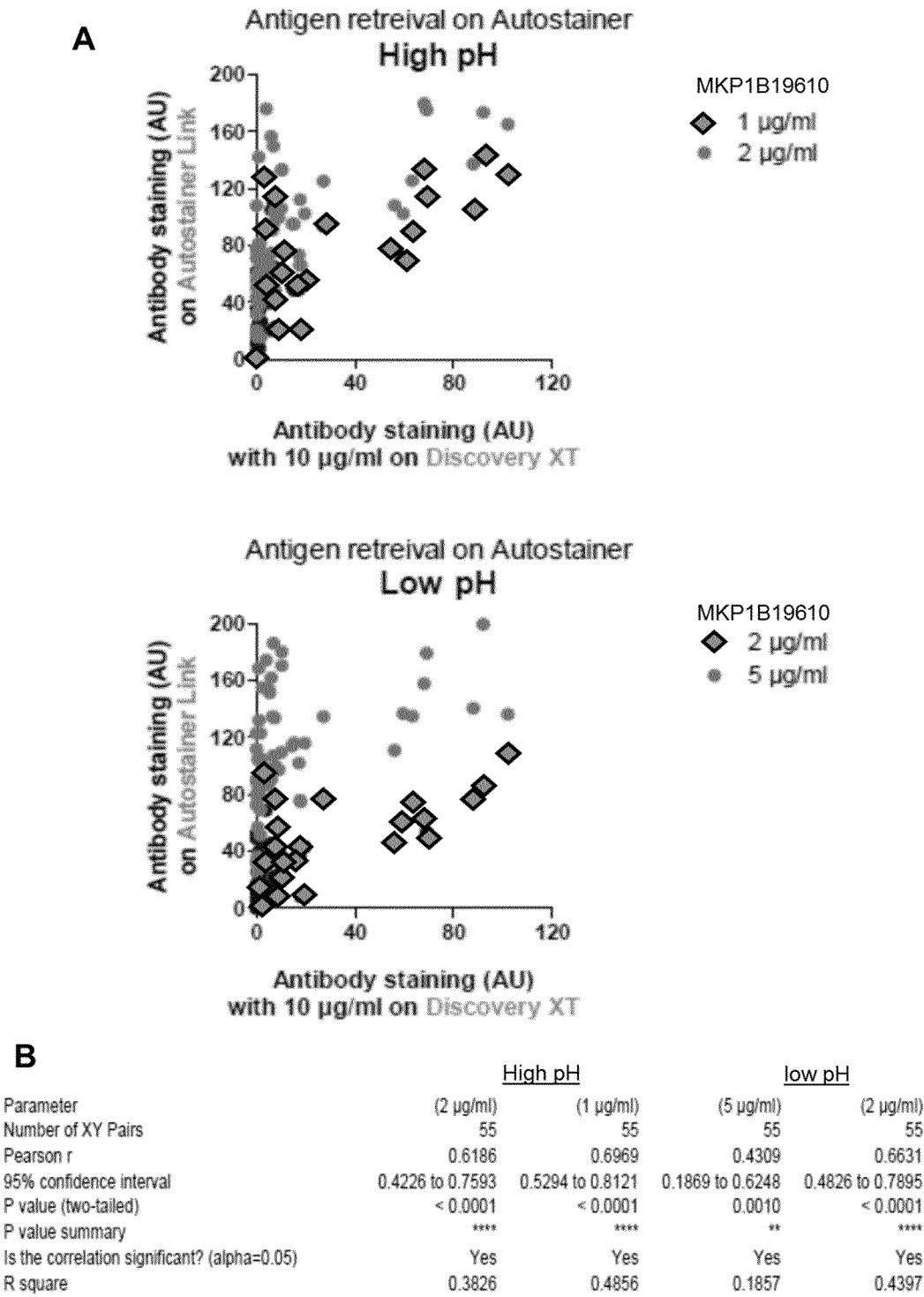
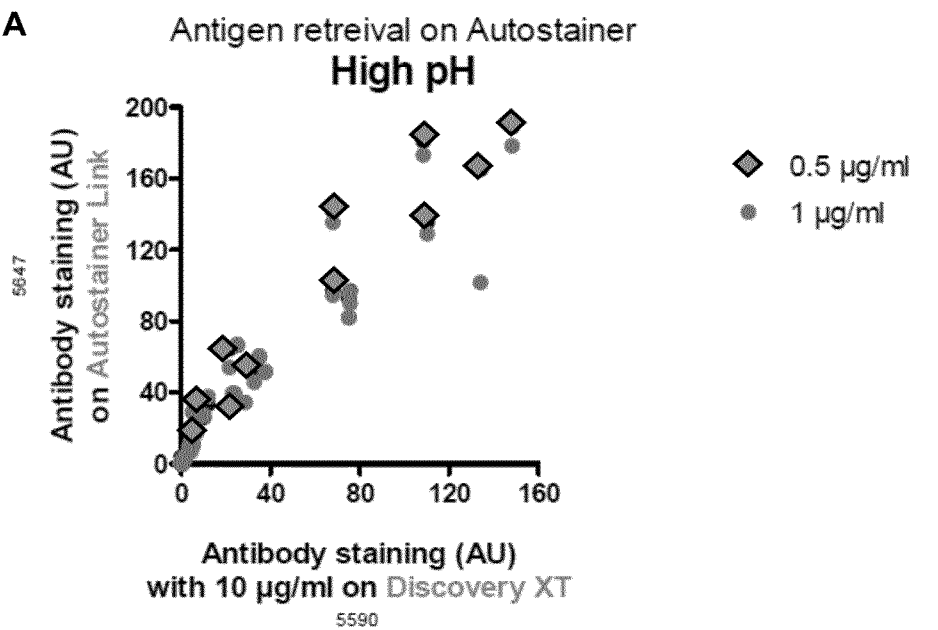


FIGURE 12

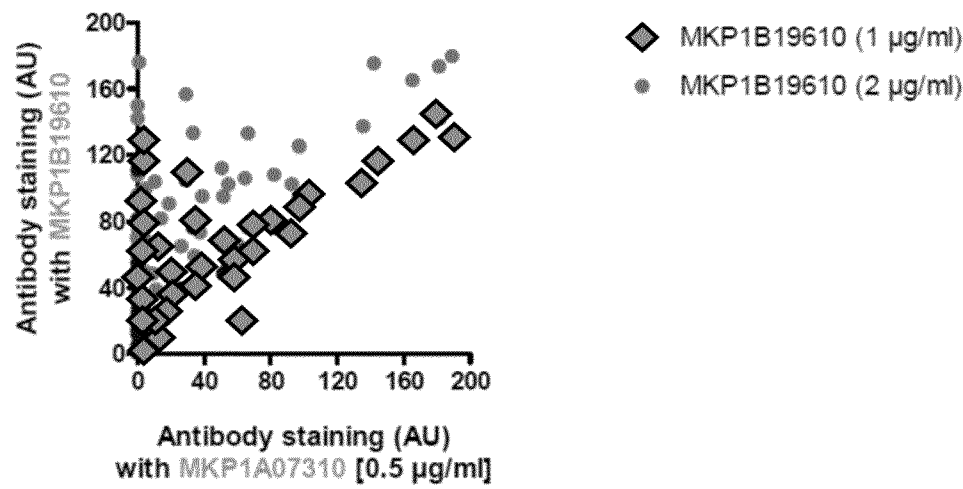


**B**

Parameter	5647 (1 µg/ml)	5647 (0.5 µg/ml)
Number of XY Pairs	55	55
Pearson r	0.9474	0.9695
95% confidence interval	0.9110 to 0.9691	0.9480 to 0.9822
P value (two-tailed)	< 0.0001	< 0.0001
P value summary	****	****
Is the correlation significant? (alpha=0.05)	Yes	Yes
R square	0.8975	0.9399

FIGURE 13

A



B

Parameter	MKP1B19610 (2µg/ml)	MKP1B19610 (1 µg/ml)
Number of XY Pairs	55	55
Pearson r	0.6503	0.7088
95% confidence interval	0.4652 to 0.7809	0.5461 to 0.8199
P value (two-tailed)	< 0.0001	< 0.0001
P value summary	****	****
Is the correlation significant? (alpha=0.05)	Yes	Yes
R square	0.4229	0.5024

FIGURE 14



## INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2017/073712

A. CLASSIFICATION OF SUBJECT MATTER  
INV. C07K16/28 G01N33/53  
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, Sequence Search, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/165422 A1 (MERCK SHARP & DOHME [US]; DOLLED-FILHART MARISA [US]; EMANCIPATOR KENN) 9 October 2014 (2014-10-09) table 2 page 27	1-50
X	JAN TRØST JØRGENSEN: "Companion diagnostic assays for PD-1/PD-L1 checkpoint inhibitors in NSCLC", EXPERT REVIEW OF MOLECULAR DIAGNOSTICS, vol. 16, no. 2, 27 November 2015 (2015-11-27), pages 131-133, XP055426614, GB ISSN: 1473-7159, DOI: 10.1586/14737159.2016.1117389 abstract ----- -/-	1-50



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

20 November 2017

Date of mailing of the international search report

14/12/2017

Name and mailing address of the ISA/

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## INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2017/073712

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/181342 A1 (SPRING BIOSCIENCE CORP [US]; HOFFMANN LA ROCHE [CH]) 3 December 2015 (2015-12-03) paragraph [0197] -----	1-50
X	JACQUELYN SMITH ET AL: "Quantitative and qualitative characterization of Two PD-L1 clones: SP263 and E1L3N", DIAGNOSTIC PATHOLOGY, vol. 11, no. 1, 18 May 2016 (2016-05-18), XP055426740, DOI: 10.1186/s13000-016-0494-2 abstract -----	1-50
A	Jian Hong ET AL: "Frequent PD-L1 expression in thymic squamous cell carcinoma of Chinese patients", Journal of Thoracic Disease J Thorac Dis, 1 January 2015 (2015-01-01), page S181, XP055426574, DOI: 10.3978/j.issn.2072-1439.2015.AB055 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4700338/pdf/jtd-07-S3-AB056.pdf [retrieved on 2017-11-20] abstract -----	1-50
A	KAUFMAN HOWARD L ET AL: "Avelumab in patients with chemotherapy-refractory metastatic Merkel cell carcinoma: a multicentre, single-group, open-label, phase 2 trial", THE LANCET ONCOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 17, no. 10, 1 September 2016 (2016-09-01), pages 1374-1385, XP029757502, ISSN: 1470-2045, DOI: 10.1016/S1470-2045(16)30364-3 the whole document -----	1-50

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2017/073712

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