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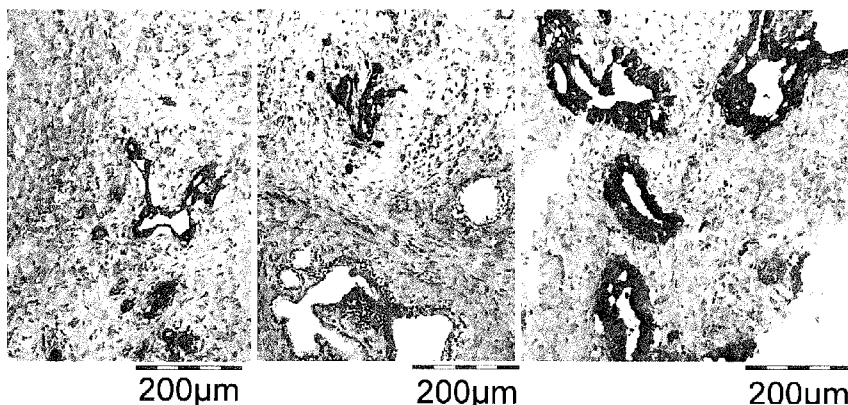
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(54) Title: ANTI-MIF IMMUNOHISTOCHEMISTRY

Figure 1A:



(57) Abstract: The present invention pertains to the specific detection of MIF, in particular oxMIF, and of anti-oxMIF antibodies in tissues. A detection method is provided which uses immunohistochemistry or immunofluorescence and wherein specific anti-oxMIF antibodies and specific idiotypic monoclonal rabbit antibodies are used.

AMENDED CLAIMS**received by the International Bureau on 27 October 2015 (27.10.2015)**

1. An immunohistochemistry (IHC) assay method for *in vitro* detection of oxMIF, wherein oxMIF is MIF which is differentially binding to antibody RAB4, RAB9 and/or RAB0, in a tissue sample of a subject, comprising the determination of the binding of a compound to oxMIF in said sample *in vitro*, wherein one or more of the following steps are carried out:
 - a) Optional Blocking step with blocking buffer and
 - b) Binding step with primary anti-oxMIF antibody without a previous fixation step, in particular either formaldehyde or acetone; wherein a washing step is carried out after binding step b) to wash away excess antibody
 - c) fixation step, wherein said fixation is carried out in formaldehyde for 10 – 25 minutes;
 - d) Incubation with secondary antibody, wherein the secondary antibody is an anti-idiotypic rabbit monoclonal antibody directed against an anti-oxMIF antibody; and
 - e) Detection of binding between the primary anti-oxMIF antibody and the secondary antibody.
2. The IHC assay of claim 1, wherein the samples is air dried, preferably for about 30 min.
3. The IHC assay of any one or more of claims 1 – 2, wherein the primary antibody is not labelled, e.g. not biotinylated, and/or is preferably comprised in a primary dilution buffer and/or wherein the primary antibody is incubated with the sample preferably for 45 to 90 minutes, more preferred for approximately 60 minutes.
4. The IHC assay of any one or more of claims 1 – 3, wherein optionally further washing steps are carried out after all further steps.
5. The IHC assay of any one or more of claims 1 – 4, wherein the detection step e) comprises or consists of a staining step and/or wherein a further (counter)staining step is carried out after the detection step, which can include the use of a tertiary antibody.
6. The IHC assay of any one or more of claims 1 – 5, wherein the (counter) staining step is carried out with hematoxylin, after step e).
7. The IHC assay of any one of the preceding claims, wherein the binding between the primary and secondary antibody is detected by fluorescence, or a directly fluorescence labelled secondary antibody (e.g. labelled anti-Ram9), preferably by use of a fluorophore labelled tertiary antibody directed against the secondary antibody.
8. The IHC assay of any one of claims 1-7, wherein said secondary antibody is a secondary monoclonal antibody selected from the group consisting of an anti-RAM9 antibody, an anti-RAM0 antibody and an anti-RAM4 antibody and is most preferred an anti-RAM9 antibody, even more preferred a rabbit anti-RAM9 antibody.
9. The IHC assay of any one of claims 1-8, wherein the primary antibody binds to oxMIF, but does not bind to redMIF.

10. The IHC assay of claim 9, wherein the differential binding is a binding to oxMIF which occurs with a K_D value of less than 100 nM, preferably less than 50 nM, even more preferred less than 10 nM and a non-binding to redMIF which is characterized by a K_D of more than 400 nM.
11. The IHC assay of any one of claims 1 to 10, wherein the primary antibodies are selected from the group consisting of oxMIF binders, like e.g. antibodies RAB4, RAB9 and/or RAB0 and/or RAM4, RAM9 and/or RAM0.
12. The IHC assay of any of the preceding claims, wherein the sample is a tissue biopsy, preferably a frozen tissue biopsy, preferably an OCT embedded section, or a core needle biopsy.
13. An idiotypic antibody, specifically binding to an anti-oxMIF antibody, preferably to an anti-RAM9 antibody.
14. The idiotypic antibody of Claim 13, wherein the antibody is characterized by an IC_{50} of ≤ 60 ng/ml in a neutralizing ELISA with immobilized MIF and RAM9.
15. An idiotypic antibody, according to Claim 13 or 14 for use in the method or kit of any one or more of the preceding claims which is characterized by SEQ ID NO: 15 and/or 16, or by SEQ ID NO: 17 and/or 18.
16. An anti-idiotypic antibody, for use in the method or kit of any one or more of the preceding claims, which is characterized by a heavy chain which has been deposited at the DSMZ with deposit No. DSM 28180 and/or by a light chain, which has been deposited at the DSMZ with deposit No. DSM 28181; or which is characterized by a heavy chain which has been deposited at the DSMZ with deposit No. DSM 28182 and/or a light chain which has been deposited at the DSMZ with a deposit no. DSM 28183.
17. A method for the detection of an anti-oxMIF antibody, preferably RAM9, RAM0 or RAM4, with the use of an idiotypic monoclonal rabbit antibody directed against said anti-oxMIF antibodies, preferably as described in any one of claims 13-16.
18. An IHC assay kit, adapted to carry out the method according to any one or more of the preceding claims; comprising
 - an anti-idiotypic antibody as described in Claim 16 and
 - an anti-oxMIF antibody.