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Published:

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(88) Date of publication of the international search report:
19 February 2009

(54) Title: ASSAY FOR DETECTION OF TRANSIENT INTRACELLULAR CA²⁺

(57) Abstract: This invention relates to a simple end point assay for detection of transient intracellular Ca²⁺ with broad applicability to many Ca²⁺ channel proteins comprising, Generation of expression constructs for the fusion proteins having the Ca²⁺/calmodulin dependent protein kinase II (CaMKII) phosphorylation sites of NR2A or NR2B subunits of N-methyl-D-aspartate receptor (NMDAR) or the voltage gated potassium channel of Drosophila (Eag) or any protein sequence which binds to the T-site of CaMKII similar to NR2B, conjugated to mitochondrial localizing signal sequence, or mutants of these sequences as described herein. Generation of mammalian expression constructs of α -CaMKII as a chimera with green fluorescent protein (GFP-D-CaMKII) or its mutants as described herein. Site-Directed mutagenesis, Transfection, Ca²⁺ stimulation, imaging and quantification of the number of cells with Ca²⁺-dependent signal, wherein, NMDA receptor activity assay, TRPV1 receptor activity assay, GluR4 receptor activity assay are performed to detect the activity Of Ca²⁺ channel proteins.

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INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC⁸: **G01N 33/50** (2006.01)

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)

IPC⁸: 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)

Wpi, Epodoc, Pubmed, Internet, TXT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Strack S, McNeill RB, Colbran RJ. "Mechanism and regulation of calcium/calmodulin-dependent protein kinase II targeting to the NR2B subunit of the N-methyl-D-aspartate receptor." J Biol Chem. 2000 Aug 4;275(31):23798-806. <i>*Whole document*</i>	1-5,7-12
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A	Praseeda M, Pradeep KK, Krupa A, Krishna SS, Leena S, Kumar RR, Cheriyan J, Mayadevi M, Srinivasan N, Omkumar RV. "Influence of a mutation in the ATP-binding region of Ca ²⁺ /calmodulin-dependent protein kinase II on its interaction with peptide substrates." Biochem J. 2004 Mar 1;378(Pt 2):391-7. <i>*Whole document*</i>	1-5,7-12
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☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
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International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Mayadevi M, Praseeda M, Kumar KS, Omkumar RV. "Sequence determinants on the NR2A and NR2B subunits of NMDA receptor responsible for specificity of phosphorylation by CaMKII." Biochim Biophys Acta. 2002 Jul 29;1598(1-2):40-5. <i>*Whole document*</i>	1-5,7-12
A	Omkumar RV, Kiely MJ, Rosenstein AJ, Min KT, Kennedy MB. "Identification of a phosphorylation site for calcium/calmodulindependent protein kinase II in the NR2B subunit of the N-methyl-D-aspartate receptor." J Biol Chem. 1996 Dec 6;271(49):31670-8. <i>*Whole document*</i>	1-5,7-12

INTERNATIONAL SEARCH REPORT

International application No. PCT/IN 2008/000370

Continuation of first sheet

Continuation No. II:

Observations where certain claims were found unsearchable

(Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 6 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 6 is unclear due to the unclear features "and cells which develop the signal for binding as described herein" and does not describe a complete technical solution for the quantification of respective calcium signals in terms of technical features and therefore had to be excluded from the search as well as the establishment for novelty, inventive step and industrial applicability.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/IN 2008/000370

Patent document cited
in search report

Publication
date

Patent family
member(s)

Publication
date

A

none