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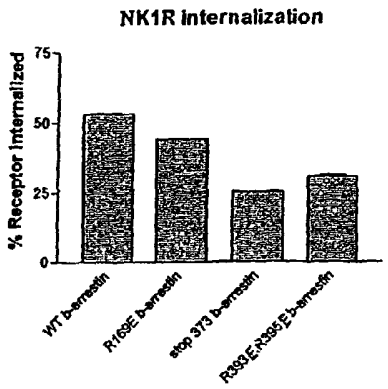
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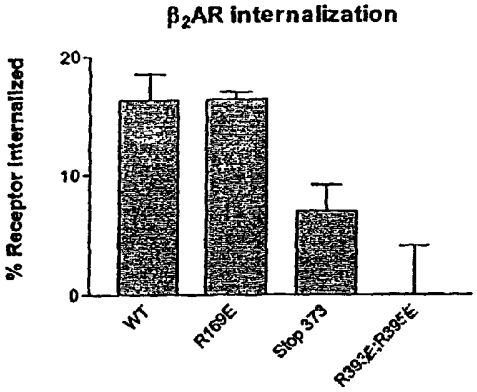
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[Continued on next page]

(54) Title: METHOD FOR ENHANCING AND PROLONGING THE BIOLUMINESCENCE RESONANCE ENERGY TRANSFER (BRET) SIGNAL IN A BRET ASSAY AND A SUBSTRATE SOLUTION FOR USE IN A BRET ASSAY





(57) Abstract: An improved BRET assay, wherein the BRET signal is enhanced and/or prolonged. The improved BRET assay comprises the steps of i) adding a substrate to a cell comprising GPCR-Rluc fusion protein and a β -arrestin-GFP fusion protein, wherein the (β-arrestin is mutated, ii) adding a ligand to obtain, if possible, a GPCR-Rluc/(β-arrestin-GFP complex, and iii) measuring a BRET signal to obtain a BRET ratio, wherein the improvement leads to an increased BRET ratio compared with the ratios obtained by use of the same process employing a β-arrestin-GFP fusion protein wherein the β-arrestin is the wild type β-arrestin, or employing a 13-arrestin-GFP fusion protein, wherein the (β-arrestin is a β-arrestin specifically mutated so that it acts on the receptor independent of the receptors phosphorylation state. The invention further relates to a stable substrate solution for use in an improved BRET assay.

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MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK 03/00687

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/566 C12Q1/48

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)
IPC 7 G01N C12Q C07K

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 practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/67106 A (MILLENNUM PHARMACEUTICALS INC) 13 September 2001 (2001-09-13) the whole document ---	1-35
X	WO 02/46763 A (NOVO NORDISK AS) 13 June 2002 (2002-06-13) the whole document ---	1-35
A	ERIK JOLY ET AL: "Bioluminescence Resonance Energy Transfer (BRET 2 TM). Principle, Applications and Products" PACKARD BIOSCIENCE, BIOSIGNAL PACKARD INC., [Online] 2001, pages 1-6, XP002267972 Retrieved from the Internet: <URL:http://www2.cbm.uam.es/confocal/Manuales/bret.pdf> [retrieved on 2004-01-26] the whole document ---	1-35

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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

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"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	Date of mailing of the international search report
12 March 2004	26.05.04

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK 03/00687

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ABRAHAM KOVOOR ET AL: "Targeted Construction of Phosphorylation-independent Beta-Arrestin Mutants with Constitutive Activity in Cells" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 11, 12 March 1999 (1999-03-12), pages 6831-6834, XP002267973 the whole document ---	1-35
A	US 5 891 646 A (FERGUSON STEPHEN S ET AL) 6 April 1999 (1999-04-06) the whole document -----	1-35

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 03/00687

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
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:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1, 11-13,18-20 and 33-35.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/ DK 03/ 00687

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1,11,12,13,18-20,33-35

   Claims: 1, 11, 12, 13, 18-20, 33-35 directed to an improved BRET assay comprising a ?-arrestin mutated so that it acts on the receptor independent of the receptors phosphorylation state.

2. Claims: 1-7,12-20,33-35

   Claims: 1-7, 12-20, 33-35 directed to an improved BRET assay by inhibiting the internalization of the GPCR-Rluc/ ?-arrestin-GFP complex.

   2.1. Claims: 1-6, 14-16, 12, 13, 18-20, 33-35 where ?-arrestin is mutated or truncated so that binding to AP2 is impaired or so that ?-arrestin does not contain any AP2 binding sites or that the AP2 binding sites are impaired in their binding to AP2.

   2.2. Claims: 1-5, 14-16, 12, 13, 18-20, 33-35 where ?-arrestin is mutated or truncated so that its binding to clathrin is impaired or so that ?-arrestin does not contain any clathrin binding sites.

   2.3. Claims: 1-3, 7, 17, 12, 13, 18-20, 33-35 where ?-arrestin is mutated to prevent the binding to phosphoinositide.

3. Claims: 1,8-10,12,13,18-20,33-35

   Claims: 1, 8-10, 12, 13, 18-20, 33-35 directed to an improved BRET assay wherein the cell comprises a further amount of G-protein coupled receptor kinase (GRK) as compared to the amount of GRK naturally present in the cell.

4. Claims: 21-32

   Claims: 21-32 directed to an improved BRET assay using a stable substrate solution.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK 03/00687

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