Title: EMISSION RATIO METRIC INDICATORS OF PHOSPHORYLATION BY C-KINASE

Abstract: A chimeric phosphorylation indicator (CPI) as provided herein can contain a donor molecule, a phosphorylatable domain, a phosphoaminoacid binding domain (PAABD), and an acceptor molecule. Where the phosphorylatable domain is phosphorylatable by protein kinase C (PKC), the CPI is a c-kinase activity reporter (CKAR). Donor and acceptor molecules may be, independently, fluorescent proteins such as non-oligomerizing fluorescent proteins. A CPI can contain a phosphorylatable polypeptide and a fluorescent protein; the phosphorylatable polypeptide may be contained within the sequence of the fluorescent protein, or the fluorescent protein may be contained within the sequence of the phosphorylatable polypeptide. The spatiotemporal properties of the PKC signal pathway may be tested with CKAR, calcium-sensing fluorophores and FRET-based translocation assays. Polynucleotides encoding such CPIs, and kits containing the indicators and/or the polynucleotides, are provided. A method of using the chimeric phosphorylation indicators to detect a kinase or phosphorytase in a sample is provided.
A. CLASSIFICATION OF SUBJECT MATTER
IPC: C12P 21/04 (2006.01)
USPC: 435/69.7
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)
U.S.: 435/69.7
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)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td>A</td>
<td>US 6,376,257 B1 (PERSECHINI) 23 April 2002 (23.05.2002), entire document.</td>
<td>1-72</td>
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</tbody>
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Further documents are listed in the continuation of Box C.

See patent family annex.

Date of the actual completion of the international search
03 August 2006 (03.08.2006)

Date of mailing of the international search report
2 Aug 2006

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Form PCT/ISA/210 (second sheet) (April 2005)
Continuation of B. FIELDS SEARCHED Item 3:
US PATENTS, JPO, EPO, STN BIOSCIENCE: MEDLINE, BIOSIS, CAPLUS.
search terms: chimeric phosphorylation indicator, cell, operative linkage, fluorescent, binding domain, phosphoaminoacid, linkers, YFP, membrane targeting domain.