Labeled Nucleoside Polyporphosphates

The present invention describes new compositions of matter in the form of labeled nucleoside polyphosphates with four or more phosphates. In addition, compositions of nucleoside polyphosphates with four or more phosphates that are substrates for nucleic acid polymerases with enhanced substrate properties and methods of using these nucleoside polyphosphates for nucleic acid detection, characterization and quantification are described. The compositions provided by this invention include nucleoside polyphosphate, dideoxynucleoside polyphosphate, or deoxynucleoside polyphosphate analogues which have colorimetric, chemiluminescent, or fluorescent moieties, mass tags or an electrochemical tags attached to the terminal phosphate. When a nucleic acid polymerase uses this analogue as a substrate, an enzyme-activatable label would be present on the inorganic polyphosphate by-product of phosphoryl transfer. Cleavage of the polyphosphate product of phosphoryl transfer via phosphate leads to a detectable change in the label attached thereon. When the polymerase assay is performed in the presence of a phosphatase, there is provided a convenient method for real-time monitoring of DNA or RNA synthesis and detection of a target nucleic acid.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12Q 1/68; C12P 19/34; C07H 21/02, 21/04, 19/00
US CL : 435/6, 91.1,91.2; 536/22.1,23.1,24.3,24.31,24.32,24.33
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. : 435/6, 91.1,91.2; 536/22.1,23.1,24.3,24.31,24.32,24.33

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>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>US 5,849,487 A (HASE et al) 15 December 1998 (15.12.98), see whole document</td>
<td>17</td>
</tr>
</tbody>
</table>

☐ 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
  "B" earlier application or patent 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 data claimed

"F" later document published after the international filing date or priority data 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: 28 January 2003 (28.01.2003)

Date of mailing of the international search report: 1 APR 2003

Name and mailing address of the ISA/US
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Jeffrey Siew
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Form PCT/ISA/210 (second sheet) (July 1998)
Continuation of B. FIELDS SEARCHED Item 3:
EAST, STN-BIOSIS, MEDLINE, CANCERLIT, BIOTECHDS, LIFESCI, CAPLUS, EMBASE
search terms: polyphosphate, sequencing, label, dye, reporter, terminal
## INTERNATIONAL SEARCH REPORT

### Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

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

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

2.  
   - Claim Nos.: 18-27  
     - 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: the method claims improperly dependent on composition claims.

3.  
   - Claim 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:

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.:  

**Remark on Protest**

- The additional search fees were accompanied by the applicant’s protest.
- No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)