Certificate of Analysis

Clontech® PCR-Select™ cDNA Subtraction Kit

Catalog No.	Amount	Lot Number
637401	7 rxns	Specified on product label.

Description

Kit for identifying cDNAs that correspond to differentially expressed sequences in one cDNA population compared with another. Enough reagents are provided for seven cDNA reactions. PCR primers are provided for 50 primary and 100 secondary PCR amplifications—enough for one control and six complete subtractions if the cDNA from each synthesis is used for tester and driver in separate subtractions.

Package Contents

Box 1:

- 5 µl Control Poly A^+ RNA (1 mg/ml)

Box 2:

- 7 µl SMARTScribe™ Reverse Transcriptase (100 units/µl)
- 10 µl cDNA Synthesis Primer (10 µM)
- 200 µl 5X First-Strand Buffer (RNase-free)
- 10 µl Dithiothreitol (DTT, 20 mM)
- 28 µl 20X Second-Strand Enzyme Cocktail
- 200 µl 5X Second-Strand Buffer
- 14 µl T4 DNA Polymerase (3 units/µl)
- 300 µl 10X Rsa I Restriction Buffer
- 12 µl Rsa I (10 units/µl)
- 21 µl T4 DNA Ligase (400 units/µl)
- 30 µl Adaptor 1 (10 µM)
- 30 µl Adaptor 2R (10 µM)
- 200 µl 5X DNA Ligation Buffer
- 1.4 ml Dilution Buffer
- 200 µl 4X Hybridization Buffer
- 50 µl PCR Primer 1 (10 µM)
- 100 µl Nested PCR primer 1 (10 µM)
- 100 µl Nested PCR primer 2R (10 µM)
- 10 µl PCR Control Subtracted cDNA
- 5 µl Control DNA
- 50 µl G3PDH 5' Primer (10 µM)
- 50 µl G3PDH 3' Primer (10 µM)
- 20 µl dNTP Mix
- 100 µl 20X EDTA/Glycogen Mix (0.2 M EDTA; 1 mg/ml glycogen)
- 480 µl NH_4OAc (4 M)
- 1 ml Sterile H_2O

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(051316)

Storage Conditions

- Store Box 1 at –70°C
- Store Box 2 at –20°C

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Shipping Conditions

- Dry ice (–70°C)

Product Documents

Documents for Clontech® products are available for download at **www.clontech.com/manuals**
The following documents apply to this product:

- Clontech PCR-Select cDNA Subtraction Kit User Manual (PT1117-1)

Quality Control Data

A sample kit was tested to ensure the quality and performance of this lot. The Control Poly A^+ RNA (from human skeletal muscle) was used for first- and second-strand cDNA synthesis. Following Rsa I digestion, 380 ng of the ds cDNA was mixed with 0.75 ng of a φX174 Hae III digest. The subtraction procedure was performed using this mixture as tester cDNA and skeletal muscle cDNA as driver. Adaptors 1 and 2R were ligated to portions of the tester cDNA, and two consecutive hybridizations were performed with tester and driver cDNAs. The Advantage® cDNA PCR Kit was used for PCR. Primary PCR was performed using PCR Primer 1; secondary PCR was performed using Nested PCR Primers 1 and 2R. The reaction products were examined by electrophoresis on a 2% agarose/EtBr gel. The bands corresponded to the Hae III-digested φX174 DNA fragments.

Notice to Purchaser

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NOTICE TO PURCHASER:

Our products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, in vitro diagnostic purposes, therapeutics, or in humans. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without prior written approval of Clontech Laboratories, Inc.

Your use of this product is also subject to compliance with the licensing requirements listed below and described on the product´s web page at http://www.clontech.com. It is your responsibility to review, understand and adhere to any restrictions imposed by these statements.

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