ProductInformation

Cyclin D Primer Set

Product No. **P8842**
Store at 0 to -20°C

Product Description

Progression through G1 phase and transition from G1 to S phase of the cell division cycle is controlled by activation of a distinct series of serine/threonine kinase complexes which are comprised of a cyclin regulatory subunit and a cyclin dependent kinase (CDK) catalytic subunit. The active cyclin-CDK complexes modify critical target proteins by phosphorylation, thus promoting cell cycle progression towards DNA replication. At least five G1-S phase cyclins have been identified: C, D1, D2, D3 and E. D and E type cyclins govern the rate of progression of mammalian cells through the first gap phase (G1) of the cell cycle and enforce the commitment of cells to replicate their DNA[2]. D-type cyclins are induced earlier than cyclin E in G1 and can form complexes with CDK-2, -4, -5, and -6. Cyclin D1 is a nuclear protein during G1 phase which disappears from the nucleus in S phase. Among the key cell cycle regulators, cyclin D1 has been implicated most strongly as a proto-oncogene in several human tumor types, including breast carcinomas.

Cyclin D PCR Primer Set includes 2 synthetic oligonucleotides to be used in PCR† reaction for the detection of Cyclin D mRNA. The "sense" oligonucleotide represents the sequence between nucleotides 106-125 and the "antisense" oligo-nucleotide represents the sequence between nucleotides 656-680 on the human cyclin D mRNA coding region.* Each primer is supplied in a separate tube and the complete set may be used for 50 amplification reactions (reaction volume of 50 µl). The primers are dried by Speed Vac and should be reconstituted in 50 µl of 1X PCR buffer to form a final working concentration of 20 µM.
* Gene bank accession No: M64349

Reagents Provided

- Cyclin D sense primer, Product No. P8967 — 1 vial
  5'-AGACCTGCGCGCCCTCGGTG-3'
- Cyclin D antisense primer, — 1 vial
  Product No. P9092,
  5'-GTAGTAGGACAGGAAGTTGTTC-3'

Reagents and Equipment Required But Not Provided
(Sigma product numbers have been given where appropriate)

- *Taq* DNA Polymerase, Product Nos. D4545 or D1806
- Deoxynucleotide Mix, Product No. D7295
  10 mM dATP, 10 mM dCTP 10 mM dGTP,
  10 mM TTP
  Dilute to a final concentration of 2.5 mM deoxynucleotides with water
- Water, Product No. W1754
- Mineral Oil, Product No. M8662
- 10X PCR Buffer, Product No. P2192 or equivalent
- DNA to be amplified
- PCR pipet tips
- 0.5 ml thin wall PCR microcentrifuge tubes

Storage

Store at 0 to -20°C. Do not store in a "frost free" freezer. Repeated freezing and thawing is **not** recommended.

Procedure

Use 1 µl of each reconstituted primer in a 50 µl PCR reaction mixture.
Recommended annealing temperature: 63°C.

Note: In order to obtain best results, determine optimal working conditions by titration test.

1. Spin down the dried primers in a microcentrifuge at maximal speed before reconstitution. Reconstitute the primers by adding 50 µl of 1X PCR buffer to each vial. Mix carefully to make sure all material is resuspended. Perform a short spin in a micro-centrifuge at maximal speed.

2. Add the following reagents to a 500 µl microcentrifuge tube in the following order:

x µl	Water (for a final volume of 50 µl)
5 µl	10X PCR Buffer
1 µl	dNTP mix (2.5 mM)
1 µl	Sense Primer
1 µl	Antisense Primer
0.5 µl	*Taq* DNA Polymerase
y µl	Template DNA (typically 10ng)
50 µl	Total volume

3. Mix gently by vortexing and briefly centrifuge to collect all components to the bottom of the tube.

4. Add 100 µl of mineral oil to the top of each tube to prevent evaporation.

5. The amplification parameters will vary depending on the primers and the thermocycler used. It may be necessary to optimize the system for individual primers, template, and thermocycler.
   Common cycling parameters are:

   a. Denature the template at 95°C for 1-2 minutes.
   b. Anneal primers at 63°C for 1 minute.
   c. Extension at 72°C for 1 minute.
   25-30 cycles of amplification are recommended.
   d. Final at 72°C for 10 minutes.
   e. Hold at 8°C.

6. The amplified DNA can be evaluated by agarose gel electrophoresis and subsequent ethidium bromide staining. Mineral oil overlay may be removed by a single chloroform extraction (1:1), recovering the aqueous phase.

The primers have been tested by RT-PCR. Their specificity has been tested on cloned plasmids encoding all the cyclins, and no cross PCR amplification was observed. The Cyclin D PCR Primer Set amplifies a 574 bp fragment of the human and hamster cyclin D transcript from cDNA prepared from 80-90% confluent cell lines.

References

1. Lukas, J., *et al.*, Mol. Cell Biol., **15,** 2600-2611 (1995)
2. Matsushimi, H., *et al.*, J. Molec. Cell Biol., 2066-2076 (1991)

†The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

5/00

Please see reverse side of the invoice or packing slip.