ProductInformation

P 9217
CYCLIN E PRIMER SET

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. Cyclin E is rate limiting for the G1/S transition. Overexpression of cyclin E accelerates the G1/S transition, decreases cell size and reduces serum requirements, but the overall generation time is unchanged since S and G2 phase become longer. Cyclin E is localized in the nucleus, and forms complexes with CDK-2 (and possibly CDK-3). These complexes have the ability to associate with pRb, to phosphorylate it and hence to accelerate the exit from G1 to S phase[1].

Cyclin E PCR Primer Set includes 2 synthetic oligonucleotides to be used in PCR† reaction for the detection of Cyclin E mRNA. The "sense" oligonucleotide represents the sequence between nucleotides 695-717 and the "antisense" oligonucleotide represents the sequence between nucleotides 1086-1110 on the human cyclin E 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: M73812

REAGENTS PROVIDED

- **Cyclin E sense primer**, Product No. P 9342 — 1 vial
  5′-GTCCTGGCTGAATGTATACATGC-3′
- **Cyclin E antisense primer**, Product No. P 9467 — 1 vial
  5′-CCCTATTTTGTTCAGACAACATGGC-3′

MATERIALS AND REAGENTS REQUIRED BUT NOT PROVIDED

- *Taq* DNA Polymerase, Product No. D 4545 or D 1806
- Deoxynucleotide Mix, Product No. D 7295
  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. W 1754
- Mineral Oil, Product No. M 8662
- 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 –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: 53 °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 microcentrifuge 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 vortex 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 53 °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 E PCR Primer Set amplifies a 415 bp fragment of the human and hamster cyclin E transcript from cDNA prepared from 80-90% confluent cell lines.

REFERENCES

1. Sherr, C.J., Cell, **73,** 1059-1065 (1993)

†The PCR process is covered by patents owned by Hoffman-LaRoche, Inc. 8/97