

Certificate of Analysis

Product Name: PCR Ready First Strand cDNA

Catalog Number: Cxxxxxxx

Shipping Condition: Dry Ice

Storage Condition: -20°C

Shelf Life: One year from the date of receipt under proper storage condition

Description

Total RNA used for cDNA synthesis is isolated by modified guanidine thiocyanate techniques. 11 µg total RNA was primed by an oligo dT primer and reverse transcribed by MMLV reverse transcriptase in 40 µl final volume. RT Reaction stopped by heating at 65°C for 10 minutes. The cDNA is in 1x RT buffer. (1x RT Buffer: 50 mM Tris-Cl, pH 8.3, 75 mM KCl, 3 mM MgCl₂, 10 mM DTT). The estimated cDNA concentration is about 2.5 ng/µl. 1 µl cDNA is good enough for one PCR reaction.

Quality Control

1. The integrity of the RNA used for cDNA synthesis is examined by visual inspection for the presence of intact bands of 18s and 28s ribosomal RNA when electrophoreses on a denaturing agarose gel. The quality and purity of total RNA were tested by spectrophotometer. A_{260/280} is between 1.8 and 2.0 (detected in 10 mM Tris-Cl, pH 7.5). The ratio of 28S/18S is ≥1.
2. The RNA used for cDNA synthesis is treated by DNase I, and is tested as DNA free RNA by PCR.
3. The synthesized cDNA was 5' selected to ensure its full length. The cDNA was used as template for PCR amplification of β-actin gene and an 838 bp β-actin band was visualized on 2% agarose gel. β-actin control primer is included. It is enough for 10 PCR reactions.

Control PCR condition

| | |
|---------------------------------|---------|
| PCR Ready First Strand cDNA | 1 µl |
| 10 x PCR Buffer | 2.5 µl |
| 2.5 mM dNTP | 4 µl |
| Control primers (25 µM) | 1 µl |
| H ₂ O, Nuclease-free | 16.3 µl |
| Taq Polymerase(5 u/µl) | 0.2 µl |

The PCR thermocycling: 94°C x 2 minutes, 1 cycle,
94°C x 30 seconds, 55°C x 30 seconds, 72°C x 30 seconds, 35 cycles
72°C x 5 minutes, 1 cycle. Then hold at 4°C.

Note: If customers failed to detect or amplify low abundant genes from CBI cDNAs, we recommend customers make their own cDNAs with CBI mRNAs as template.

Warranty: CBI is committed to providing high quality products to customers. All cDNA products passed the QC standard described in the certificate of analysis. If customers are not satisfied with any of cDNA products, it will be CBI's decision to either replace the product or to credit the full purchase price and delivery charge. All cDNA products are for Research Use Only.

FOR RESEARCH USE ONLY

APPROVED BY: 