ALPHA DIAGNOSTIC INTERNATIONAL

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Product Data Sheets

Cat # RC34-200 *Rat Tissue cDNA: Placenta* *Size: 10 Rxn*

Form *liquid* *Powder* *Store at -20C or below for 1 yr*

Includes Beta-actin control primer for 10 PCR reactions

Source of Material: Rat (Normal) Tissue: Placenta

Description

The cDNA is synthesized using total RNA extracted by modified guanidine thiocyanate method. 11 µg of total RNA was reverse transcribed by MMLV reverse-transcriptase using oligo dT primer in a 40 µl final volume. The reaction was inhibited by incubating at 65ºC 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) with a concentration of about 2.5 ng/µl. 1 µl cDNA is sufficient for one PCR reaction.

Quality Control

1. The RNA integrity was examined by visualizing intact bands corresponding to 18s and 28s ribosomal RNA after electrophoresis on a denaturing agarose gel. The total RNA quality was testified spectrophotometrically with A260/280 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 â-actin gene and an 838 bp â-actin band was visualized on 2% agarose gel. The pack includes Beta- actin control primer with a volume enough for 10 PCR reactions.

Control PCR condition

Suggested Protocol A

PCR Ready First Strand cDNA	1 µl
PCR mix	12.5 µl
Control primers (25 µM)	1 µl
H_2O, Nuclease-free	10.5µl
TOTAL	25. ul

Suggested Protocol B

Taq polymerase (5U/ul)	0.2ul
10X PCR buffer	2.5 ul
10mM dNTP	0.5 ul
H2O nuclease free	19.8 ul
control primers (5 uM)	1.0 ul
PCR Ready First Strand cDNA	1 µl
TOTAL=25 ul	

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

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

NOTES: If you are amplifying genes with multiple copies per cell, then use a target of 30 cycles. If you are amplifying genes that contain a single copy per cell, then use a target of 35 cycles. We recommend using 1 min per kb for extension. For example, a 3-min extension period is designed to amplify a 3-kb gene fragment. If the gene-specific primers have a Tm less than 70℃, then subtract 2℃ to obtain the appropriate annealing temperature.

C. Electrophoresis

Run the final PCR product on a 1.1% agarose/ethidium bromide gel alongside a suitable size marker. For the control gene, you should observe a visible band at ~900 bp when you view the gel under UV light.

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