ALPHA DIAGNOSTIC INTERNATIONAL

Product Data Sheet

Cat # HA-200			Human Adult cDNA Tissue: Placenta	**Size**: 10 Rxn
Form liquid	Powder		Store at -20C or below for 1 yr	

Source of Material: Human Placenta Tissue

Description

Total RNA used for cDNA synthesis is isolated by modified guanidine thiocyanate techniques. 11 ƒÊ g total RNA was primed by an oligo dT primer and reverse transcribed by MMLV reverse transcriptase in 40 ƒÊ l final volume. RT Reaction stopped by heating 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). The estimated cDNA concentration is about 2.5 ng/ml. 1 ƒÊ l cDNA is good enough 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

PCR Ready First Strand cDNA 1 µl
10 x PCR Buffer 2.5 µl
10 mM dNTP 0.5 µl
Control primers (5 µM) 1 µl
H2O, Nuclease-free 19.8 µl
Taq Polymerase(5 u/µl) 0.2 µl

The PCR parameters: 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 fail to detect or amplify low abundant genes using ADI cDNAs, we recommend customers make their own cDNAs using ADI 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.

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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