ALPHA DIAGNOSTIC INTERNATIONAL

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Product Data Sheets

☐ **Cat #** CHUC-565 Chicken (Normal/Adult) Universal (pool of tissue cDNA) **Size**: 10 Rxn

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

Source of Material: Chicken (Normal/Adult) Universal (pool of tissue cDNA)
Note: the age and type of tissue/tumor may change but is specified in the product data sheet

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 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. A260/280 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 human, animal, and cell line 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 1% agarose gel. β-actin control primer is included. It is enough for 10 PCR reactions.
4. The synthesized plant cDNA was used as template for PCR amplification of chloroplast gene. A 458 bp chloroplast band was visualized on 1% agarose gel. Chloroplast control primer is included. It is enough for 10 PCR reactions.

Control PCR Conditions

Taq Polymerase (5 u/ul)	0.2ul
10 x PCR Buffer	2.5ul
10 mM dNTP	0.5ul
H20, Nuclease-free	19.8ul
Control primers (5 um)	1.0ul
PCR Ready First Strand cDNA	1.0ul
Total Volume	**25.0ul**

The PCR parameters: 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 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°C, then subtract 2°C 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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