cDNA from tissue

For detials of current tissue speicfic cDNA available please contact us: info@amsbio.com

Applications: The cDNA is primed with oligo dT primer and is ideal for gene expression analysis by PCR amplification of known genes, characterization of alternative splicing of mRNA, gene cloning and target sequencing and verification of genetic mutation.

Storage Conditions: Store at -20ºC. One year from the date of receipt under storage condition.

Quality Control: The PCR-Ready cDNA was functionally examined by:

- The integrity of the RNA used for cDNA synthesis is tested 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.
- The RNA used for cDNA synthesis is treated by DNase I and is tested as DNA free RNA by PCR.
- 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 ß-actin gene and an 838 bp ß-actin band was visualized on 1% agarose gel. ß-actin control primer is included. It is enough for 10 PCR reactions.
- 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.

Description: The PCR ready first strand cDNA was synthesized from high quality RNA isolated by modified guanidine thiocyanate method. 10µg total RNA was primed by oligo dT primer and reverse transcribed by MMLV. RT reaction stopped by heating at 65 ºC for 10 minutes. The cDNA is in 1x RT buffer (1x RT buffer contain 50mM Tris-Cl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10mM DTT). The estimated cDNA concentration is about 5.5ng/µl. 1µl cDNA is good enough for one PCR reaction. The 5' end of human clathrin cDNA (a 6 kb gene) has been amplified by PCR from all of these cDNAs.

Control PCR component is as follow (2 options):

Components	Volume (µl /reaction)	Final concentration
PCR Mix (Cat#.: L5051100) (contains Taq polymerase, dNTPs, reaction buffer and Taq DNA polymerase enhancer)	12.5	
H_2O, nuclease-free	10.5	-
Control primers	1	5 µM
cDNA	1	
Total volume	25 µl	

Components	Volume (µl /reaction)	Final concentration
Taq Polymerase (Cat#.:L7051001 or L7051200)	0.2	5u/µl
PCR buffer	2.5	10X
dNTP (Cat#.:K6011105)	0.5	10 mM
H_2O, nuclease-free	19.8	-
Control primers	1	5µM
cDNA	1	
Total volume	25 µl	

Control PCR condition is as follow:

	Denaturation	35 CYCLES			Final Extension	Hold
		Denaturation	Annealing	Extension		
Time	2 min.	30 sec.	30 sec.	30 sec.	5 min.	-
Temperature	94 ºC	94 ºC	55 ºC	72 ºC	72 ºC	4ºC