Product Specification

Gene Expression, Microarrays, Real Time Quantitative PCR, cDNA cloning, PCR amplification

Guinea Pig First Strand cDNA

Store at -20^{0}C
For research use only. Not for use in diagnostic procedures for clinical purposes.

❑ 10-2100-05	Pooled cDNA	5μg	❑ 10-2106-05	Skeletal muscle	5μg
❑ 10-2101-05	Brain	5μg	❑ 10-2107-05	Lung	5μg
❑ 10-2102-05	Heart	5μg	❑ 10-2108-05	Spleen	5μg
❑ 10-2103-05	Liver	5μg	❑ 10-2109-05	Ovary	5μg
❑ 10-2104-05	Kidney	5μg	❑ 10-2110-05	Pancreas	5μg
❑ 10-2105-05	Intestine	5μg	❑ 10-2111-05	Eye	5μg
			❑ 10-2112-05M	Male adipose tissue	5μg

Background

First strand cDNA is useful for amplifying a particular cDNA using PCR. The PCR reaction must be optimized using varying amounts of the cDNA. This optimization is particularly important when the target mRNA species is of low abundance. The protocol given is for amplifying β-actin as a control to validate the quality of the 'first strand cDNA' supplied. The PCR conditions to amplify the target cDNA will be based on the primers selected. It should be noted that specific sequence primers as well as degenerate sequence primers can be used successfully to amplify the target sequence.

The first strand cDNA has been prepared from freshly obtained Hartley strain guinea pig tissue and appropriately frozen during transportation. RNA was extracted using the widely used and published method (1). Oligo dT has been used to prime the synthesis of the first strand using Moloney Murine leukemia Virus (MMLV) Reverse Transcriptase. The amount supplied is sufficient for at least 50 amplifications. Each lot is tested for amplification of β-actin cDNA.

Material Supplied

1.	**First strand cDNA**	**5μg (lyophilized)**
2.	**β-actin control PCR mix**	**200μl**

Reconstitution

The 'First strand cDNA' is supplied lyophilized. Spin the tube briefly before opening to make sure that the DNA is collected at the bottom of the tube. Reconstitute it in 50μl sterile water.

The β-actin control PCR mix is ready to use with the supplied first strand cDNA.

Amplification of target sequence cDNA

Amplification of target sequence cDNA requires optimization using varying amounts of the first strand cDNA based on the abundance of the mRNA. Generally 1-5μl of the first strand cDNA is sufficient as the template. It is a good strategy to amplify short segments (200-300 bp) initially, and depending on the amplification results, longer segments could be attempted for amplification. Another proven method is to peform nested PCR using the amplification product of the first PCR.

β-actin control PCR

Set up two PCR reaction tubes for the control. To each tube add 50μl of the supplied β-actin control PCR mix. To each of these tubes add 2μl and 4μl of the reconstituted first strand cDNA. Add 2.5 units of Taq polymerase preferably after initial denaturation, using the 'hot-start' method.

PCR* reaction (see Appendix for Details)

PCR Profile

Denaturation	94oC	30 sec.
Annealing	55oC	30 sec.
Elongation	72oC	1 min.

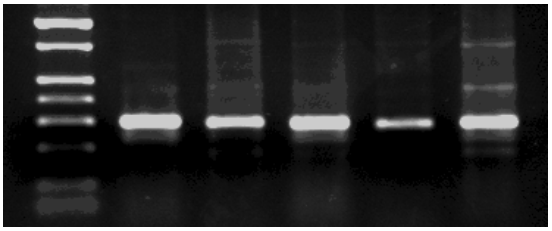
30 cycles, 7 min. 72oC extension, 4oC soak.

Electrophoresis

Load samples to 1.5% agarose gel. Run at 90 mAmps for 2.5 hrs.

Results

An amplified fragment of 289 bp. Lane 1 is molecular weight markers. Lanes 2-6 are β-actin control PCR product from brain, liver, intestine, skeletal muscle and spleen.



References

1. Chomczynski,P. and Sacchi, N. (1987) Anal. Biochem. 162:156-159.

Appendix

PCR Premix preparation
Typical Premix

	/50 µl rxn	**/1ml**
10 x PCR Buffer	4.5 µl	100µl
dNTP mix (2.5mM each)	4 µl	100µl
Primer Mix (10 pmol/µl each) (25 pmol of each primer/50µl)	2.5 µl	63µl
Sterile water	34 µl	737µl
Total	45 µl	1ml

Nucleotide Dilution
Stock: 100 mM; Prepare a final diluted 2.5 mM solution
Preparation

Each 100 mM dNTP	125 µl (Total 500 µl)
Water	4.5 ml
Total volume	5.0ml

Taq Premix *(per 50µl reaction, scale up as required)*

10 x PCR Buffer	0.5µl
Taq polymerase (2.5 units)	0.25µl
Sterile water	4.25µl
	5µl/rxn.

PCR reaction (50µl)

Diluted DNA(100ng/µl)	1 µl
PCR premix	45 µl
Taq premix	5 µl

PCR products post-processing
1. For oil layered PCR only. Add 200µl of $CHCl_3$ to each tube, vortex and spin.
2. Transfer the upper aqueous layer to a fresh eppendorf tube, add 1/10 volume of 3M NaAc (pH 5.2), and 2 volumes of absolute ethanol, precipitate DNA at -80^{0}C for 10 minutes.
3. Spin, rinse the DNA pellet with 700µl of 75% ethanol and dry the pellet in the speedvac.
4. Dissolve the pellet in adequate amount of TE.

Ordering Information

First Strand cDNA

Product	Catalog No.	Size	Price $
Guinea pig first strand pooled cDNA	10-2100-05	5µg	425.00
Guinea pig first strand cDNA, Brain	10-2101-05	5µg	425.00
Guinea pig first strand cDNA, Heart	10-2102-05	5µg	425.00
Guinea pig first strand cDNA, Liver	10-2103-05	5µg	425.00
Guinea pig first strand cDNA, Kidney	10-2104-05	5µg	425.00
Guinea pig first strand cDNA, Intestine	10-2105-05	5µg	425.00
Guinea pig first strand cDNA, Skeletal muscle	10-2106-05	5µg	425.00
Guinea pig first strand cDNA, Lungs	10-2107-05	5µg	425.00
Guinea pig first strand cDNA, Spleen	10-2108-05	5µg	425.00
Guinea pig first strand cDNA, Ovaries	10-2109-05	5µg	425.00
Guinea pig first strand cDNA, Pancreas	10-2110-05	5µg	425.00
Guinea pig first strand cDNA, Eye	10-2111-05	5µg	425.00
Guinea pig first strand cDNA, Male adipose tissue	10-2112-05M	5µg	425.00

Please inquire about custom cDNA synthesis

Related Products

Omni-Array™ mRNA amplification kits

Product	Catalog No.	Size	Price $
Omni-Array ™ Sense strand mRNA amplification kit, 100ng Version	08-0011-10	10 rxns.	495.00
Omni-Array ™ Antisense strand mRNA amplification kit, 100ng Version	08-0021-10	10 rxns.	495.00

Omni-mRNA™ amplified pooled reference mRNA

Quantity supplied 50 µg in 25 µg x 2 tubes is sufficient for direct hybridization of 20 microarrays

Product	Catalog No.	Size	Price $
Human Omni-mRNA™ amplified pooled reference mRNA	08-0100-50	50µg (25µg x 2 tubes)	395.00
Mouse Omni-mRNA™ amplified pooled reference mRNA	08-0200-50	50µg (25µg x 2 tubes)	395.00
Rat Omni-mRNA™ amplified pooled reference mRNA	08-0300-50	50µg (25µg x 2 tubes)	395.00
Guinea Pig Omni-mRNA™ amplified pooled reference mRNA	08-2100-50	50µg (25µg x 2 tubes)	395.00

**The polymerase chain reaction (PCR) process is covered by patents owned by Hoffmann-La Roche. A license to perform is automatically granted by the use of authorized reagents.

Prices subject to change without notice
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