

# Product Sheet

## First Strand cDNA

<input type="checkbox"/> 10-2101-05 Brain	5µg
<input type="checkbox"/> 10-2102-05 Heart	5µg
<input type="checkbox"/> 10-2103-05 Liver	5µg
<input type="checkbox"/> 10-2104-05 Kidney	5µg
<input type="checkbox"/> 10-2105-05 Intestine	5µg

## Guinea Pig

<input type="checkbox"/> 10-2106-05 Skeletal muscle	5µg
<input type="checkbox"/> 10-2107-05 Lung	5µg
<input type="checkbox"/> 10-2108-05 Spleen	5µg
<input type="checkbox"/> 10-2109-05 Ovary	5µg
<input type="checkbox"/> 10-2110-05 Pancreas	5µg

Shipped at ambient temperature. Store at -20°C

**For research use only.** Not for use in diagnostic procedures for clinical purposes.

## 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  $\beta$ -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 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  $\beta$ -actin cDNA.

## Material Supplied

1. **First strand cDNA** 5µg (lyophilized)
2.  **$\beta$ -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  $\beta$ -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 perform nested PCR using the amplification product of the first PCR.

\*\*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.

All Gene Link products are for research use only.

## $\beta$ -actin control PCR

Set up two PCR reaction tubes for the control. To each tube add 50µl of the supplied  $\beta$ -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

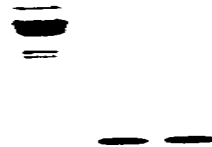
Denaturation	94oC	30 sec.
Annealing	55oC	30 sec.
Elongation	72oC	1 min.
30 cycles, 7 min. 72oC extension, 4oC soak.		

## 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 2-3 are  $\beta$ -actin control PCR product.



## 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)	2.5 µl	63µl
(25 pmol of each primer/50µl)		
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<sub>3</sub> 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°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

First strand cDNA is useful for amplifying a particular cDNA using PCR. β-actin PCR mix is included with the product 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. Each lot is tested for amplification of β-actin cDNA.

The first strand cDNA is prepared from freshly obtained tissue and appropriately frozen during transportation. RNA is extracted using the widely used and published method (Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162:156-159). Oligo dT is 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.

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

Please inquire about custom cDNA synthesis

### GENEMER™

Product	Size	Catalog No.	Price, \$
Sickle Cell SC2/SC5 primer pair	10nmoles	40-2001-10	100.00
RhD (Rh D gene exon 10 specific)	10nmoles	40-2002-10	100.00
Rh EeCc (Rh Ee and Cc exon 7 specific)	10nmoles	40-2003-10	100.00
Fragile X (spanning triple repeat region)	10nmoles	40-2004-10	100.00
Gaucher 1226G mutation specific	10nmoles	40-2005-10	100.00
Gaucher 1448C mutation specific	10nmoles	40-2006-10	100.00
Gaucher 84GG mutation specific	10nmoles	40-2007-10	100.00
Gaucher IVS2 mutation specific	10nmoles	40-2008-10	100.00
Cystic Fibrosis ΔF508	10nmoles	40-2009-10	100.00
Cystic Fibrosis G542X	10nmoles	40-2010-10	100.00
Cystic Fibrosis W1282X	10nmoles	40-2011-10	100.00
Cystic Fibrosis G551D/R553X	10nmoles	40-2012-10	100.00
Cystic Fibrosis N1303K	10nmoles	40-2013-10	100.00
Cystic Fibrosis CT3849	10nmoles	40-2014-10	100.00
SRY (sex determining region on Y)	10nmoles	40-2020-10	100.00
X aliphoid repeat	10nmoles	40-2021-10	100.00
Y aliphoid repeat	10nmoles	40-2022-10	100.00

Please inquire about other GENEMER™ not listed here

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Prices subject to change without notice

All Gene Link products are for research use only.



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