Product Sheet

Custom First Strand cDNA

Shipped at ambient temperature. Store at -20^{o}C

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

Gene Link provides custom first strand cDNA synthesis service for RT-PCR. Each lot is tested for amplification of β-actin cDNA.

The first strand cDNA is prepared from tissue provided by the customer or Gene Link will locate the appropriate tissue source. The amount supplied is sufficient for at least 50 amplifications.

Background

First strand cDNA is useful for amplifying a particular cDNA using PCR. The PCR reaction has to 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 could be used successfully to amplify. Each lot is tested for amplification of β-actin cDNA.

The first strand cDNA will be prepared from tissue provided by the customer or Gene Link will locate a source for the appropriate tissue. RNA is extracted using the widely used and published method (1). 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.

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 to amplify. Another proven method is to do 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 this tube 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.

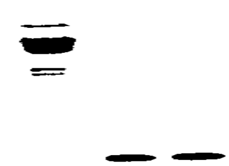
30 cycles, 7 min. 72oC extension, 4oC soak.

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 β-actin control PCR product.



References

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

**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.

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Gene Link ™

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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
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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^{O}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 could 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
Custom first strand cDNA; customer supplied tissue	5μg	10-2000-XX	$2,550.00

Please see catalog for in stock cDNA

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 FibrosisCT3849	10nmoles	40-2014-10	100.00
SRY (sex determining region on Y)	10nmoles	40-2020-10	100.00
X alphoid repeat	10nmoles	40-2021-10	100.00
Y alphoid repeat	10nmoles	40-2022-10	100.00

Please inquire about other GENEMER™ not listed here

Prices subject to change without notice

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