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

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 5 µg (lyophilized)
2. β-actin control PCR mix 200 µl

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.

Thermal Cycler Files

Amplification Profile

The following amplification profile has been optimized for specific product amplification using the supplied Genemer™.

Program the following thermal cycler files.

1. Hot Start

Hot Start		
Step	Time & Temperature	Cycles
Initial Denaturation	95 °C for 5 minutes	1
Annealing	60 °C Hold Infinity	Hold
Comments: Add Taq premix while on hold.		

2. Amplification File

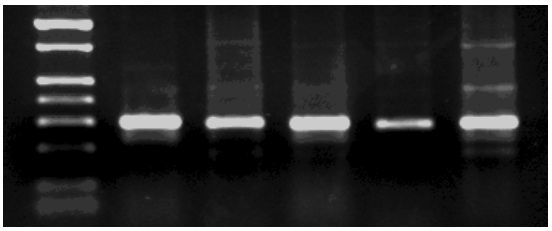
Amplification File			
Step	Temperature	Time	Cycles
Denaturation	94 °C	30 sec.	30
Annealing	55 °C	30 sec.	
Elongation	72 °C	60 sec.	
Fill in Extension	72 °C	7 minutes	1
Hold	4 °C	Infinity	Hold

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**.**

Ordering Information

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

Related Products

Omni-cDNA™ First Strand cDNA		
Product	**Catalog No.**	**Size**
Human Omni-cDNA™ first strand pooled cDNA	10-0100-05	5μg
Mouse Omni-cDNA™ first strand pooled cDNA	10-0200-05	5μg
Rat Omni-cDNA™ Rat first strand pooled cDNA	10-0300-05	5μg
Guinea Pig Omni-cDNA™ first strand pooled cDNA	10-2100-05	5μg

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**
Human Omni-mRNA™ amplified pooled reference mRNA	08-0100-25	25 μg
Mouse Omni-mRNA™ amplified pooled reference mRNA	08-0200-25	25 μg
Rat Omni-mRNA™ amplified pooled reference mRNA	08-0300-25	25 μg
Guinea Pig Omni-mRNA™ amplified pooled reference mRNA	08-2100-25	25 μg

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

All Gene Link products are for research use only Current pricing are posted at http://www.genelink.com/