



Product Specification

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

Omni-cDNA™ Pooled First Strand cDNA

Store at -20°C
For research use only

<input type="checkbox"/>	Human Omni-cDNA™ Cat No. 10-0100-05	<input type="checkbox"/>	Mouse Omni-cDNA™ Cat No. 10-0200-05	<input type="checkbox"/>	Rat Omni-cDNA™ Cat No. 10-0300-05	<input type="checkbox"/>	Guinea Pig Omni-cDNA™ Cat No. 10-2100-05
--------------------------	--	--------------------------	--	--------------------------	--------------------------------------	--------------------------	---

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 pooled and or amplified mRNA obtained from different tissues. These are not from cultured cell lines. The various tissues vary, but are representative of different organs and tissue types. These include lung, heart, brain, spleen, skeletal muscle, smooth muscle, ovaries, pancreas, liver and kidney. There is lot to lot variation but an overall representation of tissue type is maintained. Oligo dT has been used to prime the synthesis of the first strand using Moloney Murine leukemia Virus (MMLV) Reverse Transcriptase or AMV 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 μ g (lyophilized)
2. β -actin control PCR mix 200 μ l

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 50 μ l 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 1-5 μ l 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.

β -actin control PCR

Set up two PCR reaction tubes for the control. To each tube add 20 μ l of the supplied β -actin control PCR mix. To each of these tubes add 2 μ l and 4 μ l of the reconstituted first strand cDNA. Add 1 unit of Taq polymerase preferably after initial denaturation, using the 'hot-start' method.

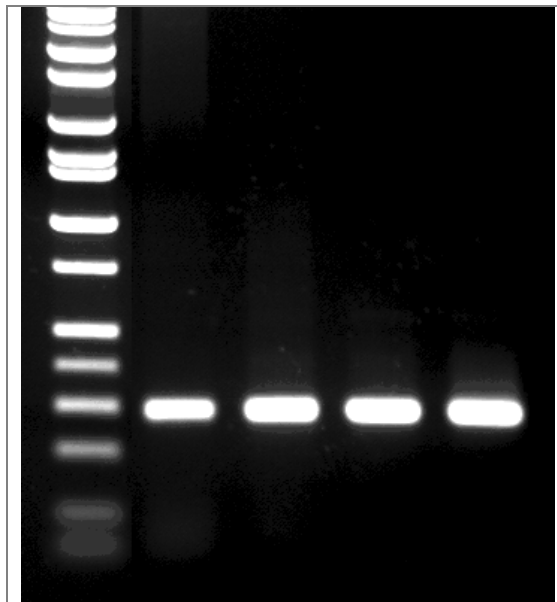
PCR Profile

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

Electrophoresis

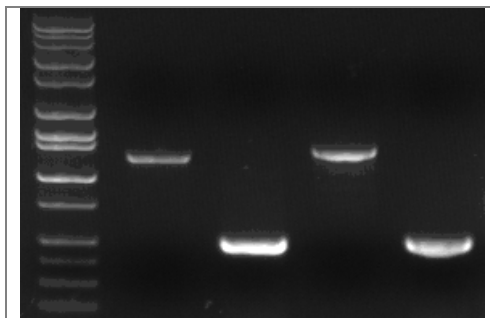
Load 5 μ l samples to 1.5% agarose gel. Run at 90 mAmps for ~2 hrs.

Results



β -actin amplified fragment of 289 bp. Lane 1 is molecular weight markers. Lanes 2-5 are β -actin control PCR product from guinea pig, human, mouse and rat pooled first strand Omni-cDNA™.

Omni-cDNA™ pooled first strand size distribution is from ~5kb to 200bp. These can also be used for cloning mRNA of interest by RT-PCR. A 1.3 kb and a ~500bp amplified cDNA fragment of p53 is shown in the figure below.



p53 cDNA amplification from human Omni-mRNA™ pooled reference mRNA. Lane 1, molecular weight markers; lanes 2 and 4, ~1.3kb 5' end fragment of p53; lane 3 and 5, ~500 bp of middle portion of p53. Lanes 2-3 and 4-5 represent reproducible different preparations

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/>