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

❑	Human Omni-cDNA™ Cat No. 10-0100-05	❑	Mouse Omni-cDNA™ Cat No. 10-0200-05	❑	Rat Omni-cDNA™ Cat No. 10-0300-05	❑	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 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 20μl of the supplied β-actin control PCR mix. To each of these tubes add 2μl and 4μl 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.

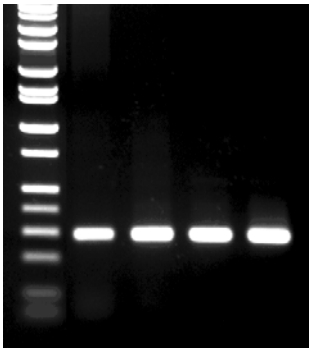
30 cycles, 7 min. 72°C extension, 4°C soak.

Electrophoresis

Load 5μl 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™.



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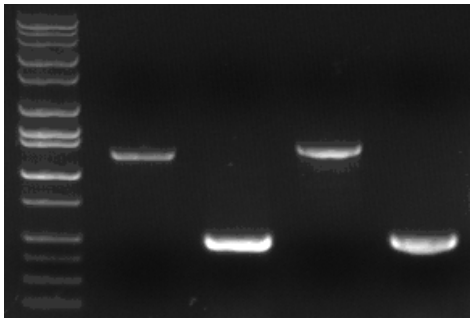
Omni-cDNA™ pooled first strand size distribution is from ~5kb to 200bp. These can also be used for cloning mRNA of interest by RT-PCR. A 1.3 kb and a ~500bp amplified cDNA fragment of p53 is shown in the figure.



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

Omni-cDNA™ First Strand cDNA				
Product		**Catalog No.**	**Size**	**Price $**
Omni-cDNA™	Human first strand pooled cDNA	10-0100-05	5μg	425.00
Omni-cDNA™	Mouse first strand pooled cDNA	10-0200-05	5μg	425.00
Omni-cDNA™	Rat first strand pooled cDNA	10-0300-05	5μg	425.00
Omni-cDNA™	Guinea Pig first strand pooled cDNA	10-2100-05	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-Array ™ Sense strand mRNA amplification kit, 5ng Version	08-0015-10	10 rxns.	695.00
Omni-Array ™ Antisense strand mRNA amplification kit, 5ng Version	08-0025-10	10 rxns.	695.00

Omni-mRNA™ amplified pooled reference mRNA Quantity supplied of 25 μg is sufficient for direct hybridization of 20 microarrays			
Product	**Catalog No.**	**Size**	**Price $**
Human Omni-mRNA™ amplified pooled reference mRNA	08-0100-25	25μg	395.00
Mouse Omni-mRNA™ amplified pooled reference mRNA	08-0200-25	25μg	395.00
Rat Omni-mRNA™ amplified pooled reference mRNA	08-0300-25	25μg	395.00
Guinea Pig Omni-mRNA™ amplified pooled reference mRNA	08-2100-25	25μg	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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