Product Specification

RT-PCRmer™
Human & Rat ß-actin Control PCR Mix

Catalog No. 40-1002-00 Size: 200 µl
Ship: Ice pack Storage: -20°C

Locus	Primers	Fragment Size	Exon	Tm
Human & Rat ß-actin	ß-actin 1/2	289 bp	3	55°C
Note: This primer set will amplify a fragment of 289 bp from human and rat cDNA.				
The fragments can be distinguished from rat or human source by digestion with Pvu II; the rat amplified 289 bp fragment is digested to give a 132 and 157 fragments whereas the human amplified fragment is not digested due to the absence of the Pvu II.				

Description

ß-actin is ubiquitously expressed and serves as a positive control for northern, other expression studies and as controls for measuring cDNA synthesis efficiency by reverse transcription and as controls for mRNA (cDNA) quantitative expression studies.

The human and Rat ß-actin PCR mix contains RT-PCRmer™ (Catalog Number 40-1001-10), PCR buffer and dNTP's. It is ready to use by just adding template cDNA and Taq polymerase.

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

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.

PCR Profile

Denaturation	94oC	30 sec.
Annealing	55oC	30 sec.
Elongation	72oC	1 min.

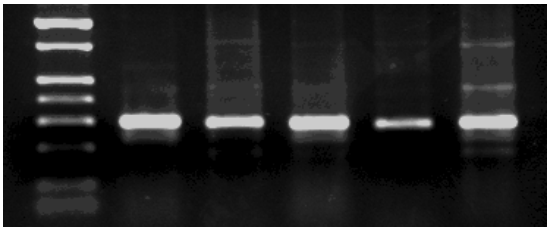
30 cycles, 7 min. 72oC extension, 4oC soak.

Electrophoresis

Load samples to 1.5% agarose gel. Run at 90 mAmps for 1.5 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.



Reference

1. du Breuil, R. M., Patel, J.M. and Mendelow, B.V. (1993) PCR methods and applications. 3:57-59.

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