Store at -20°C

#5037

SimpleChIP® Human γ-Actin Promoter Primers

☑ 500 µl
(250 PCR reactions)

Cell Signaling TECHNOLOGY®

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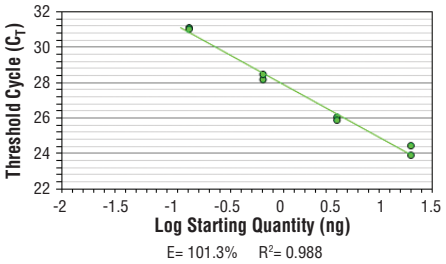
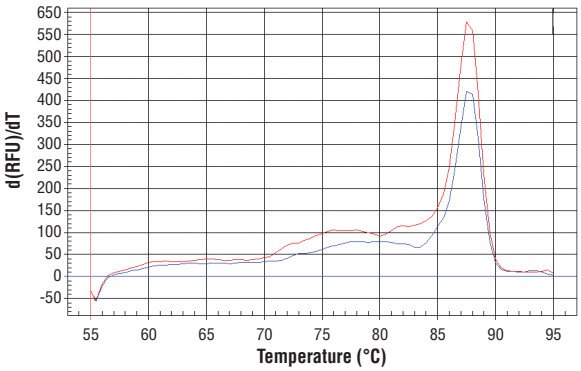
For Research Use Only. Not For Use In Diagnostic Procedures.

Applications	Species Cross-Reactivity	Primer Anneal/Extension	PCR Product Length
ChIP	H	65°C	79 bp

Description: SimpleChIP® Human γ-Actin Promoter Primers contain a mix of forward and reverse PCR primers that are specific to a region of the human γ-actin promoter. These primers can be used to amplify DNA that has been isolated using chromatin immunoprecipitation (ChIP). Primers have been optimized for use in SYBR® Green quantitative real-time PCR and have been tested in conjunction with SimpleChIP® Enzymatic Chromatin IP Kits #9002 and #9003 and ChIP-validated antibodies from Cell Signaling Technology®. The γ-actin gene is actively transcribed in all cell types and its promoter is highly enriched for histone modifications associated with active transcription, such as histone H3 Lys4 tri-methylation and general histone acetylation. This gene promoter shows very low levels of histone modifications associated with heterochromatin, such as histone H3 Lys9 or Lys27 tri-methylation.



SimpleChIP® Human γ-Actin Promoter Primers were tested on DNA isolated from cross-linked cells using the SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. Real-time PCR was performed in duplicate on a serial dilution of 2% total input DNA (20 ng, 4 ng, 0.8 ng, and 0.16 ng) using a real-time PCR detection system and SYBR® Green reaction mix. The PCR amplification efficiency (E) and correlation coefficient (R^2) were calculated based on the corresponding threshold cycle (C_T) of each dilution sample during 40 cycles of real-time PCR (95°C denaturation for 15 sec, 65°C anneal/extension for 60 sec).



PCR product melting curves were obtained for real-time PCR reactions performed using SimpleChIP® Human γ-Actin Promoter Primers. Data is shown for both duplicate PCR reactions using 20 ng of total DNA. The melt curve consists of 80 melt cycles, starting at 55°C with increments of 0.5°C per cycle. Each peak is formed from the degradation of a single PCR product.

Storage: Supplied in nuclease-free water at a concentration of 5 µM (each primer is at a final concentration of 5 µM). Store at -20°C.

Directions for Use:

1. Label the appropriate number of PCR tubes or PCR plates compatible with the model of real-time PCR machine to be used. PCR reactions should be performed in duplicate and should include a tube with no DNA to control for contamination, and a serial dilution of a 2% total input chromatin DNA (undiluted, 1:5, 1:25, 1:125), which is used to create a standard curve and determine amplification efficiency.

2. Add 2 µl of the appropriate ChIP DNA sample to each tube or well of the PCR plate.

3. Prepare a master PCR reaction mix as described below. Add enough reagents for two extra reactions to account for loss of volume. Add 18 µl of the master PCR reaction mix to each PCR reaction tube or well of the PCR plate.

Reagent	Volume for 1 PCR Reaction (20 µl)
Nuclease-free H_2O	6 µl
5 µM SimpleChIP® Primers	2 µl
2X SYBR® Green Reaction Mix	10 µl

4. Start the following PCR reaction program:
   a. Initial Denaturation: 95°C for 3 min
   b. Denaturation: 95°C for 15 sec
   c. Anneal and Extension: Primer-specific temp. for 60 sec
   d. Repeat steps b and c for a total of 40 cycles.

5. Analyze quantitative PCR results using software provided with the real-time PCR machine.

SYBR® Green is a registered trademark of Molecular Probes, Inc.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.