

# SimpleChIP® Human $\beta$ -Actin Promoter Primers



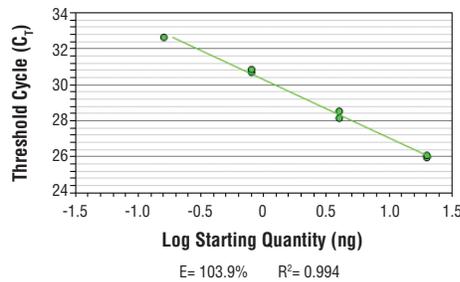
**Orders** ■ 877-616-CELL (2355)  
orders@cellsignal.com  
**Support** ■ 877-678-TECH (8324)  
info@cellsignal.com  
**Web** ■ www.cellsignal.com

rev. 06/29/17

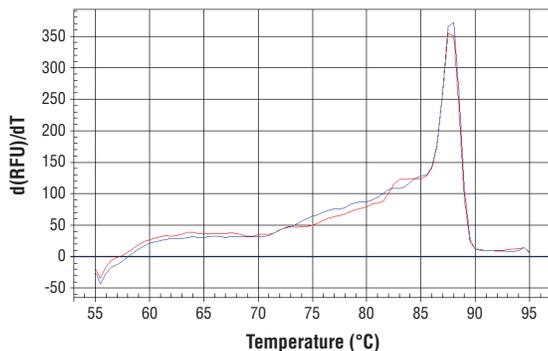
**For Research Use Only. Not For Use In Diagnostic Procedures.**

Applications	Species Cross-Reactivity	Primer Anneal/Extension	PCR Product Length
ChIP	H	65°C	148 bp

**Description:** SimpleChIP® Human  $\beta$ -Actin Promoter Primers contain a mix of forward and reverse PCR primers that are specific to a region of the human  $\beta$ -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  $\beta$ -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  $\beta$ -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<sup>2</sup>) were calculated based on the corresponding threshold cycle (C<sub>t</sub>) 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  $\beta$ -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  $\mu$ M (each primer is at a final concentration of 5  $\mu$ M). 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  $\mu$ l 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  $\mu$ l of the master PCR reaction mix to each PCR reaction tube or well of the PCR plate.
- | Reagent                        | Volume for 1 PCR Reaction (20 $\mu$ l) |
|--------------------------------|--|
| Nuclease-free H <sub>2</sub> O | 6 $\mu$ l                              |
| 5 $\mu$ M SimpleChIP® Primers  | 2 $\mu$ l                              |
| 2X SYBR® Green Reaction Mix    | 10 $\mu$ l                             |
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.