tore at -20°C

SimpleChIP® Mouse PER1 Intron 1 Primers

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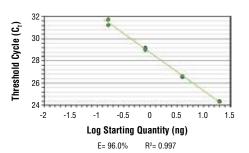
Entrez-Gene ID #18626 UniProt ID #035973

New 07/14

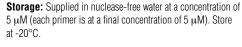
For Research Use Only. Not For Use In Diagnostic Procedures.

Applications	Species Cross-Reactivity	Primer Anneal/Extension	PCR Product Length
ChIP	M	65°C	249 bp

Description: SimpleChIP® Mouse PER1 Intron 1 Primers contain a mix of forward and reverse PCR primers that are specific to intron 1 of the mouse period circadian clock 1 gene. 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®.



SimpleChIP® Mouse PER1 Intron 1 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 (R²) were calculated based on the corresponding threshold cycle (C_7) of each dilution sample during 40 cycles of real-time PCR (95°C denaturation for 15 sec, 65°C anneal/extension for 60 sec).

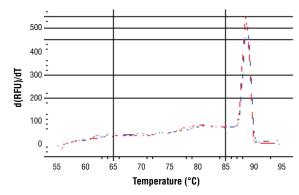


Directions for Use:

- 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 μ I of the master PCR reaction mix to each PCR reaction tube or well of the PCR plate.

ReagentVolume for 1 PCR Reaction (20 μl)Nuclease-free H_2O 6 μl5 μM SimpleChIP® Primers2 μl2X SYBR® Green Reaction Mix10 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.



PCR product melting curves were obtained for real-time PCR reactions performed using SimpleChIP® Mouse PER1 Intron 1 Primers. Data is shown for 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.

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