

SimpleChIP® Human NR1D1 Promoter Primers



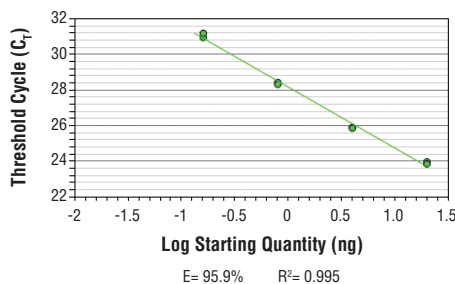
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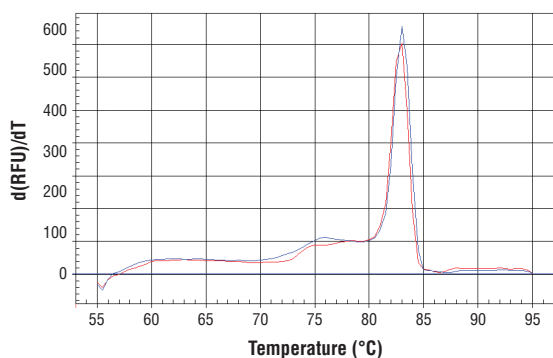
For Research Use Only. Not For Use In Diagnostic Procedures.

Applications	Species Cross-Reactivity	Primer Anneal/Extension	PCR Product Length
ChIP	H	65°C	102 bp

Description: SimpleChIP® Human NR1D1 Promoter Primers contain a mix of forward and reverse PCR primers that are specific to a region of the human nuclear receptor subfamily 1, group D, member 1 (NR1D1) 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 *NR1D1* gene encodes for the transcriptional repressor Rev-erb α , an orphan nuclear receptor that regulates cell proliferation, differentiation, and circadian rhythms in multiple tissues and organ systems.



SimpleChIP® Human NR1D1 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 (R²) 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 NR1D1 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 μ M (each primer is at a final concentration of 5 μ 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 μ 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 μ 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 μ l)
Nuclease-free H ₂ O	6 μ l
5 μ M SimpleChIP® Primers	2 μ l
2X SYBR® Green Reaction Mix	10 μ 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.

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