

SimpleChIP® Human I κ B α Promoter Primers

✓ 500 μ l
(250 PCR reactions)



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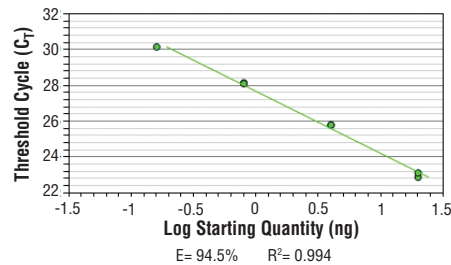
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rev. 02/21/17

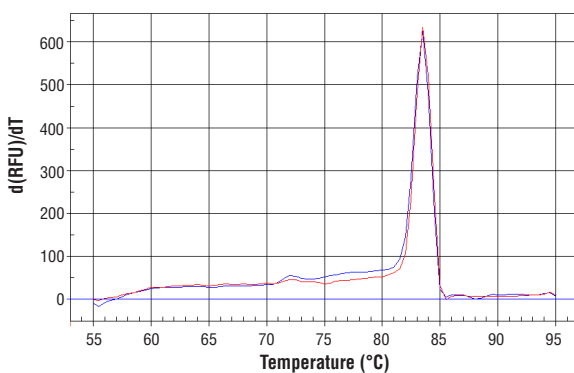
For Research Use Only. Not For Use In Diagnostic Procedures.

Applications	Species Cross-Reactivity	Primer Anneal/Extension	PCR Product Length
ChIP	H	65°C	176 bp

Description: SimpleChIP® Human I κ B α Promoter Primers contain a mix of forward and reverse PCR primers that are specific to a region of the human I κ B α promoter bound by NF- κ B proteins. 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 I κ B α gene is downregulated by NF- κ B members during inflammation signaling.



SimpleChIP® Human I κ B α 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® I κ B α 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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