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#14023

SimpleChIP® Human HSP70 Intron 1 Primers

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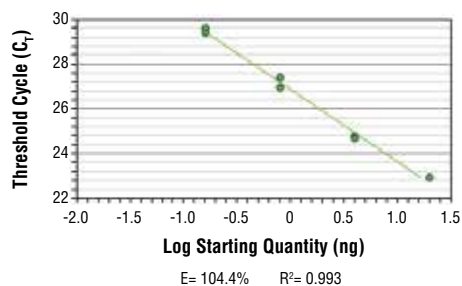
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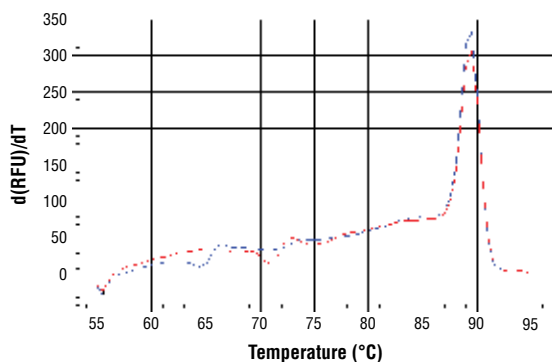
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Applications ChIP	Species Cross-Reactivity* H	Primer Anneal/Extension 65°C	PCR Product Length 115 bp
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Description: SimpleChIP® Human HSP70 Intron 1 Primers contain a mix of forward and reverse PCR primers that are specific to intron 1 of the human heat shock protein 70 (HSP70) 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®. The molecular chaperone HSP70 maintains protein homeostasis by interacting with newly synthesized and denatured unfolded proteins, preventing irreversible aggregation and catalyzing the refolding of their substrates in an ATP- and co-chaperone-dependent manner.



SimpleChIP® Human HSP70 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_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 HSP70 Intron 1 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 ₂ O	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.

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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: 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.