Store at -20°C

SimpleChIP[®] Human CTGF **Promoter Primers**

#1492

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Entrez-Gene ID #1490

UniProt ID #P29279

New 01/15

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

	Applications ChIP	Species Cross-Re H	activity	Primer Anneal/Extensio 65°C	on PCR Product Lengt 112 bp
contain a l specific to DNA that l tion (ChIP Green qua conjunctic #9002 and	ion: SimpleChIP® Human CT mix of forward and reverse PC the CTGF gene. These prime has been isolated using chron). Primers have been optimize intitative real-time PCR and ha on with SimpleChIP® Enzymat d #9003 and ChIP-validated a Technology [®] .	CR primers that are rs can be used to amplify natin immunoprecipita- ed for use in SYBR® ave been tested in ric Chromatin IP Kits	32 30 28 26 24 -2	-1.5 -1 -0.5 0 Log Starting Qua E= 102.3% R ²	0.5 1.0 1.5 ntity (ng)

SimpleChIP® Human CTGF 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_{τ}) of each dilution sample during 40 cycles of real-time PCR (95°C denaturation for 15 sec, 65°C anneal/ extension for 60 sec).

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 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 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 µl)

Nuclease-free H ₂ 0	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.

800 700 _ į, 600 1 500 -1 d(RFU)/d1 400 300 200 100 0 -100 -1. 55 60 65 70 75 80 85 90 95 Temperature (°C)

PCR product melting curves were obtained for real-time PCR reactions performed using SimpleChIP® Human CTGF 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.

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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 AII—all species expected Species enclosed in parentheses are predicted to react based on 100% homology

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