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## SimpleChIP® Human MX1 Promoter Primers

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> Entrez-Gene ID # 4599 UniProt ID # P20591

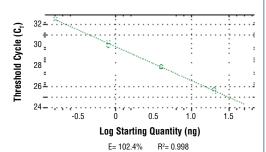
500 µl (250 PCR reactions)

New 04/15

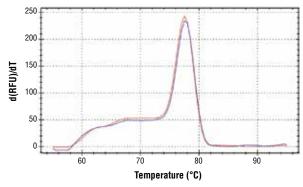
## For Research Use Only. Not For Use In Diagnostic Procedures.

Applications ChIP	Species Cross-Reactivity	Primer Anneal/Extension	PCR Product Length
	Н	65°C	81 bp

**Description:** SimpleChIP® Human MX1 Promoter Primers contain a mix of forward and reverse PCR primers that are specific to a region of the human myxovirus resistance protein 1 (MX1) 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®.



SimpleChIP® Human MX1 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 ( $R^2$ ) 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).



PCR product melting curves were obtained for real-time PCR reactions performed using SimpleChIP® Human MX1 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  $5~\mu M$  (each primer is at a final concentration of  $5~\mu M$ ). 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  $\mu l$  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  $\mu$ I 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  $\rm H_2O$  6  $\mu \rm H$  5  $\mu \rm M$  SimpleChIP® Primers 2  $\mu \rm H$  2X SYBR® Green Reaction Mix 10  $\mu \rm H$ 

- 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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