**SimpleChIP® Human α Satellite Repeat Primers**

- **500 μl** (250 PCR reactions)

**Applications**

- ChIP

**Species Cross-Reactivity**

- H

**Primer Anneal/Extension**

- 66°C

**PCR Product Length**

- 182 bp

**Description:** SimpleChIP® Human α Satellite Repeat Primers contain a mix of forward and reverse PCR primers that are specific to the human α satellite repeat element. 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 human α satellite repeat element is found in pericentromeric regions of chromosomes and shows hallmarks of heterochromatin including histone H3 Lys9 di- and tri-methylation, as well as HP1 binding.

**PCR product melting curves were obtained for real-time PCR reactions performed using SimpleChIP® Human α Satellite Repeat 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.**

**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, 1:1, 1:2, 1:4, 1:8, and 1:16) 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 (Ct) of each dilution sample during 40 cycles of real-time PCR (95°C denaturation for 15 sec, 60°C anneal/extension for 60 sec).

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 H2O: 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.

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

**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.

**Applications Key:**

- **ChIP**—Chromatin Immunoprecipitation
- **IF**—Immunofluorescence
- **FI**—Flow cytometry
- **ELISA-Peptide**—Enzyme-linked immunosorbent assay (ELISA)-Peptide

**Species Cross-Reactivity Key:**

- **H**—human
- **M**—mouse
- **R**—rat
- **Hm**—hamster
- **Mm**—mink
- **C**—chicken
- **Dm**—D. melanogaster
- **X**—Xenopus
- **Z**—zebrafish
- **B**—bovine

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