Universal qPCR Master Mix #88989  10 μl

UniProt ID
®
Human ASXL1
Plus Enzymatic
®
O  6 μl
Primer Anneal/Extension
Universal qPCR Master Mix #88989 and have
®
®
Primers  2 μl
-®
®
Human ASXL1 Upstream Primers.
®
®
®
65°C
®
PCR Product Length
-®
95 bp

PCR product melting curves were obtained for real-time PCR reactions performed using SimpleChIP® Human ASXL1 Upstream 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. SimpleChIP® Human ASXL1 Upstream Primers were tested on DNA isolated from cross-linked cells using the SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9002. 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 SimpleChIP® Universal qPCR Master Mix #88989. 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, 65°C anneal/extension for 60 sec).

**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.
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.

**Reagent**

<table>
<thead>
<tr>
<th>Volume for 1 PCR Reaction (20 μl)</th>
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<tbody>
<tr>
<td>Nuclease-free H₂O</td>
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<tr>
<td>5 μM SimpleChIP® Primers</td>
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<tr>
<td>2X SimpleChIP® Universal qPCR Master Mix #88989</td>
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**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.

**Species Cross-Reactivity:**

- H — human
- M — mouse
- R — rat
- Hm — hamster
- Mm — 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.