

Certificate of Analysis

ChIPAb+ LEF1

Catalog # 17-604

Lot # R0708G0053

Product Description: Every lot of the ChIPAb+ line of antibodies is individually validated for chromatin precipitation, in order to guarantee successful ChIP assays every time. Each antibody includes a control primer set for performance confirmation. LEF1 antibody and the negative control antibody (mouse normal IgG) can be used to demonstrate that the LEF1 antibody is functionally validated in the precipitation of LEF1 associated chromatin.

The qPCR primers included flank the LEF1/TCF binding site in human c-myc promoter.

Quantity: 25 assays per kit. ~4 µg per chromatin immunoprecipitation.

(Dependent upon biological context)

Storage and Stability: Stable for 2 years at -20°C from date of shipment.

Kit Components

Anti-LEF1 (mouse IgG₁), Cat.# CS200635. 1 vial containing **100 µg** of thiophilic and size exclusion chromatography purified mouse IgG₁ in **34 µL** of phosphate buffered saline with sodium azide. Store at -20°C. The antibody is made against amino acid residues 1-85 of human LEF1 and can recognize human and mouse LEF1. It does not cross-react with TCF-3 or TCF-4.

Normal Mouse IgG, Cat.# CS200621 . One vial containing **125 µg** of normal mouse IgG in **125 µL** volume. Store at -20°C.

ChIP primer mix, Cat.# CS200601. 1 vial containing **75 µL** of 5 µM of each control primer specific for human c-myc promoter. Store at -20°C.

FOR: CCC AAA AAA AGG CAC GGAA
REV: TAT TGG AAA TGC GGT CAT GC

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

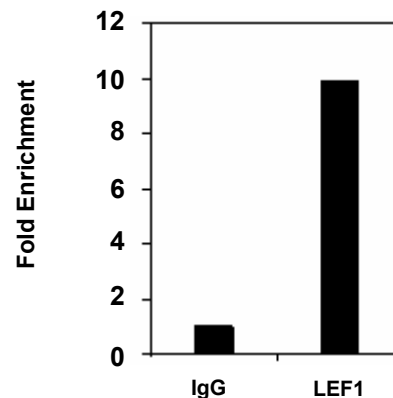
Quality Control Testing

ChIP Analysis:

Sonicated chromatin prepared from 3x10⁶ HT29 cells (ChIPable Chromatin Cat.# 12-705) was subjected to chromatin immunoprecipitation using 4 µg of either the negative control antibody, Mouse Normal IgG (Cat.# CS200621), or mouse Anti-LEF1 (Cat.# CS200635) and the Magna ChIP™ G kit (Cat.# 17-611) Rapid Protocol (2 hour IP). Successful enrichment of LEF1 associated DNA fragments was verified by qPCR using ChIP Primers c-myc , (Cat.# CS200601) flanking the human c-myc promoter that contains LEF1 binding sites (Figure 1).

Please refer to the EZ-Magna ChIP™ G (Cat.# 17-409) or EZ-ChIP™ (Cat.# 17-371) kit protocols for experimental details.

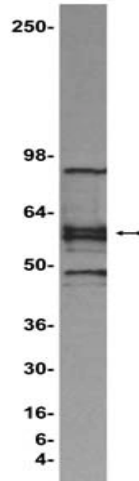
Figure 1:



Immunoblot Analysis:

Jurkat cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-LEF1 (1 µg/mL). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system (Figure 2). Arrow indicates LEF1 (55-57 kDa).

Figure 2:



qPCR Protocol

qPCR run parameters and reagent setup are listed below. Preparing qPCR master mix is recommended for multiple PCR reactions. We suggest performing triplicates for qPCR of each individual ChIP reaction.

qPCR reagent assembly for 1 reaction:

ChIP final product	5 µL
ddH ₂ O	6.5 µL
Sybr-Green Master Mix	12.5 µL
Primer mix	1 µL
<u>Total</u>	<u>25 µL</u>

qPCR parameters:

Initial Denaturation 94°C 10 min	
Denature 94°C 20 sec	} 50 times
Anneal and Extension: 60°C 1 min	

References:

1. Riese, J., et al., *Cell* 88: 777-787, 1997.
2. Giese, K., et al. *Proc. Natl. Acad. Sci. USA*, 94: 12845-12850, 1997.
3. Behrens, J., et al. *Nature*, 382: 638-642, 1996.
4. Giese, K. and Grosschedl, R. *EMBO J.*, 12: 4667-4676, 1993.

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