

Certificate of Analysis

ChIPAb+ Sp1

Catalog # 17-601

Lot # R0708G0050

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. Sp1 antibody and the negative control antibody (rabbit normal IgG) can be used to demonstrate that the Sp1 antibody is functionally validated in the precipitation of Sp1 associated chromatin.

The qPCR primers included flank the Sp1 binding site in human DHFR 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-Sp1 (Rabbit polyclonal IgG), Cat.# CS200631. 1 vial containing **100 µg** of protein A purified IgG in **100 µL** of 0.014 M phosphate buffer, pH 7.6, 0.175 M NaCl, 0.07% sodium azide and 30% glycerol. Store at -20°C. The Sp1 antibody is made against full length human Sp1 protein and can recognize Sp1, MW 105 kDa and 95 kDa, of human, mouse and rat origins.

Normal Rabbit IgG, Cat.# PP64B. One vial containing **125 µg** of normal rabbit IgG in **125 µL**. Store at -20°C.

ChIP primers DHFR, Cat.# CS200599. 1 vial containing **75 µL** of 5 µM of each control primer specific for human dihydrofolate reductase (DHFR) promoter. Store at -20°C.

FOR: TCG CCT GCA CAA ATA GGG AC

REV: AGA ACG CGC GGT CAA GTT T

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

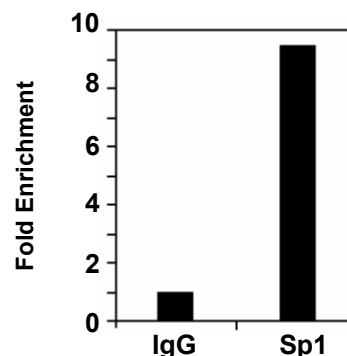
Quality Control Testing

ChIP Analysis:

Sonicated chromatin prepared from 3x10⁶ HeLa cells (ChIPable Chromatin Cat.# 12-687) was subjected to chromatin immunoprecipitation using 4 µg of either the negative control antibody, Rabbit Normal IgG (Cat.# PP64B), or Rabbit Anti-Sp1 (Cat.# CS200631) and the Magna ChIP™ A kit (Cat.# 17-610) Rapid Protocol (2 hour IP). Successful enrichment of Sp1 associated DNA fragments was verified by qPCR using ChIP Primers DHFR, (Cat.# CS200599) flanking the human DHFR promoter that contains a Sp1 binding site (Figure 1).

Please refer to the EZ-Magna ChIP™ A (Cat.# 17-408) or EZ-ChIP™ (Cat.# 17-371) kit protocols for experimental details.

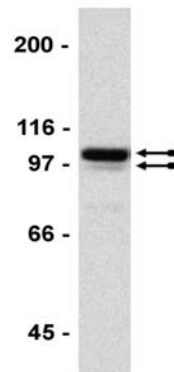
Figure 1:



Immunoblot Analysis:

HeLa nuclear extract was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Sp1 (0.2 µg/mL). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system (Figure 2). Arrows indicate Sp1 (~105 and 95 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	

General References:

1. Marban, C, *et al* (2007). Recruitment of chromatin-modifying enzymes by CTIP2 promotes HIV-1 transcriptional silencing. *EMBO J* **26**: 412-23.
2. Bouwman, P, *et al* (2000). Transcription factor Sp3 is essential for post-natal survival and late tooth development. *EMBO J* **19**: 655-61.
3. Doetzlhofer, A, *et al* (1999). Histone deacetylase 1 can repress transcription by binding to Sp1. *Mol Cell Biol* **19**: 5504-11.

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