Anti-SP1 Antibody [JF0950] - BSA and Azide free

HA750330

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, ChIP, IP
Molecular Wt:	Predicted band size: 81 kDa
Clone number:	JF0950

Description: Sp1 is a sequence-specific transcription factor that recognizes GGGGCGGGGC and closely related sequences, which are often referred to as GC boxes. Sp1 was initially identified as a HeLa cell-derived factor that selectively activates in vitro transcription from the SV40 promoter and binds to the multiple GC boxes in the 21-bp repeated elements in SV40. The sequence specificity of DNA binding is conferred by Zn (II) fingers, whereas a different region of Sp1 appears to regulate the affinity of DNA binding. Sp1 belongs to a subgroup of transcription factors that are phosphorylated upon binding to promoter sequences. Evidence suggests that the early growth response gene, Erg-1 (also known as Zif268 or NGF1-A), may downregulate certain mammalian gene promoters by competing with Sp1 for binding to an overlapping binding motif. The gene encoding human Sp1 maps to chromosome 12q13.1.

Immunogen: Synthetic peptide within Human SP1 aa 544-588 / 785.

Positive control: HeLa cell lysate, 293T cell lysate, Jurkat cell lysate, U-2 OS cell lysate, COS-1 cell lysate, HeLa, human breast cancer tissue, human colon cancer tissue, human lung tissue, mouse lung tissue, rat lung tissue, MCF-7.

Subcellular location: Nucleus, Cytoplasm.

Database links: SwissProt: P08047 Human | O89090 Mouse | Q01714 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:100-1:500
IF-Tissue	1:100-1:500
IHC-P	1:200-1:1,000
FC	1:500-1:1,000
ChIP	Use 0.5~2 μg for 25 μg of chromatin.
IP	1-2μg/sample

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

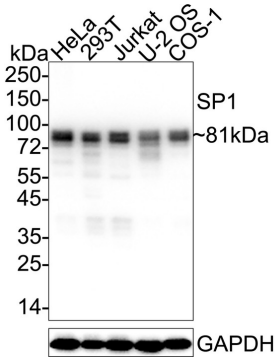

Fig1: Western blot analysis of SP1 on different lysates with Rabbit anti-SP1 antibody (HA750330) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: 293T cell lysate
Lane 3: Jurkat cell lysate
Lane 4: U-2 OS cell lysate
Lane 5: COS-1 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 81 kDa
Observed band size: 81 kDa

Exposure time: 4 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750330) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

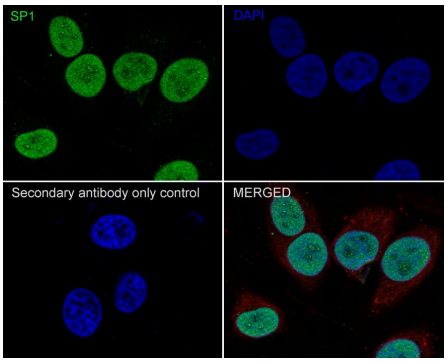

Fig2: Immunocytochemistry analysis of HeLa cells labeling SP1 with Rabbit anti-SP1 antibody (HA750330) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-SP1 antibody (HA750330) at 1/200 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/200 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

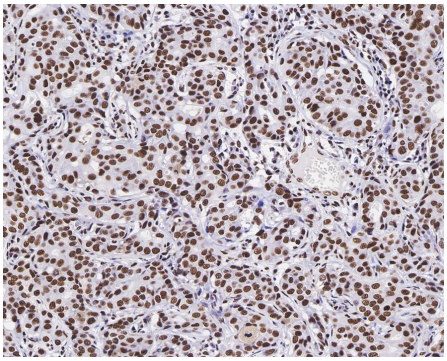

Fig3: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-SP1 antibody (HA750330) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750330) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

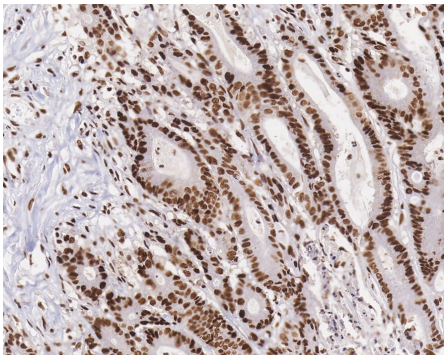

Fig4: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-SP1 antibody (HA750330) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750330) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

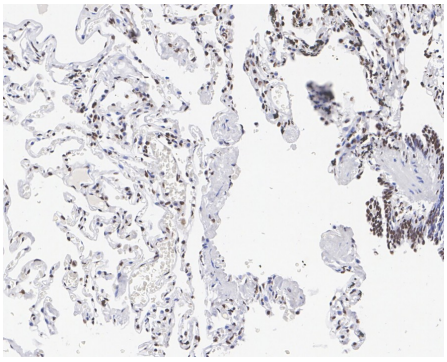

Fig5: Immunohistochemical analysis of paraffin-embedded human lung tissue with Rabbit anti-SP1 antibody (HA750330) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750330) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

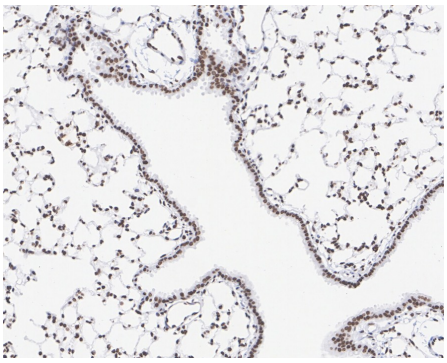

Fig6: Immunohistochemical analysis of paraffin-embedded mouse lung tissue with Rabbit anti-SP1 antibody (HA750330) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750330) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

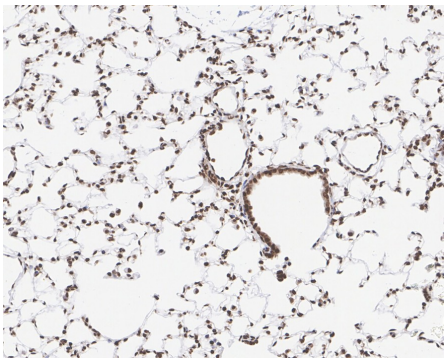

Fig7: Immunohistochemical analysis of paraffin-embedded rat lung tissue with Rabbit anti-SP1 antibody (HA750330) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750330) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

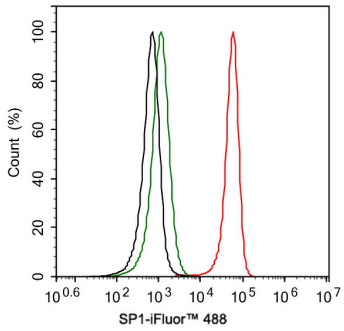

Fig8: Flow cytometric analysis of MCF-7 cells labeling SP1.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750330, 1ug/ml) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

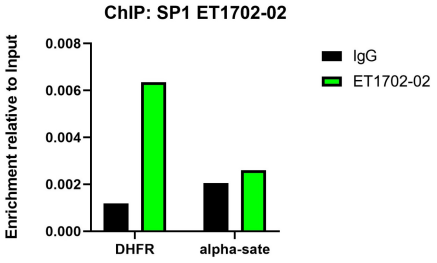

Fig9: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells with SP1 (HA750330) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

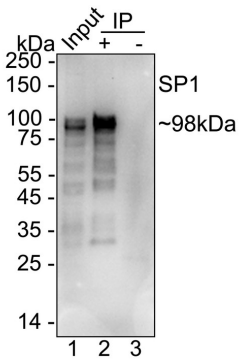

Fig10: SP1 was immunoprecipitated from 0.2 mg HeLa cell lysate with HA750330 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA750330 at 1/1,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: HA750330 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of HA750330 in HeLa cell lysate

Blocking/Dilution buffer: primary antibody dilution (K1803)
Exposure time: 6 seconds; ECL: K1801

Background References

1. Guaraldo M et al. Characterization of human mitochondrial ferritin promoter: identification of transcription factors and evidences of epigenetic control. Sci Rep 6:33432 (2016).
2. Deng X et al. Protein arginine methyltransferase 5 functions as an epigenetic activator of the androgen receptor to promote prostate cancer cell growth. Oncogene:1-9 (2016).