Anti-Phospho-NF-κB p65 (S529) Antibody [SP07-00]

ET1604-27

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 60 kDa
Clone number:	SP07-00

Description: Proteins encoded by the v-Rel viral oncogene and its cellular homolog, c-Rel, are members of a family of transcription factors that include the two subunits of the transcription factor NκB (p50 and p65) and the Drosophila maternal morphogen, dorsal. Both proteins specifically bind to DNA sequences that are the same or slight variations of the 10 bp κB sequence in the immunoglobulin κ light chain enhancer. This same sequence is also present in a number of other cellular and viral enhancers. The DNA binding activity of NFκB is activated and NFκB is subsequently transported from the cytoplasm to the nucleus in cells exposed to mitogens or growth factors. cDNAs encoding precursors for two distinct proteins of the same size have been described, designated p105 and p100. The p105 precursor contains p50 at its N-terminus and a C-terminal region that when expressed as a separate molecule, designated pdI, binds to p50 and regulates its activity.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser529 of Human NF-kB p65.

Positive control: HeLa treated with 100nM Calyculin A and 20ng/mL TNF-α for 15 minutes cell lysate, human lung tissue, human lung squamous cell carcinoma tissue, Daudi.

Subcellular location: Nucleus, Cytoplasm.

Database links: SwissProt Q04206 Human

Recommended Dilutions:

WB	1:1,000
IHC-P	1:1,000
FC	1:50
IP	Use at an assay dependent concentration.

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% SodiumAzide.

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

Purity: Protein A affinity purified.

Images

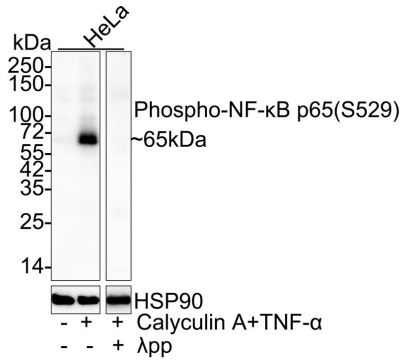

Fig1: Western blot analysis of Phospho-NF-κB p65 (S529) on different lysates with Rabbit anti-Phospho-NF-κB p65 (S529) antibody (ET1604-27) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 100nM Calyculin A and 20ng/mL TNF-α for 15 minutes cell lysate
Lane 3: HeLa treated with 100nM Calyculin A and 20ng/mL TNF-α for 15 minutes cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 µg/Lane.

Predicted band size: 60 kDa
Observed band size: 65 kDa

Exposure time: 43 seconds;

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 (ET1604-27) 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.

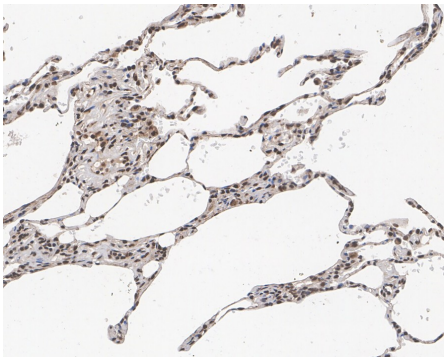

Fig2: Immunohistochemical analysis of paraffin-embedded human lung tissue with Rabbit anti-Phospho-NF-κB p65 (S529) antibody (ET1604-27) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (ET1604-27) 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.

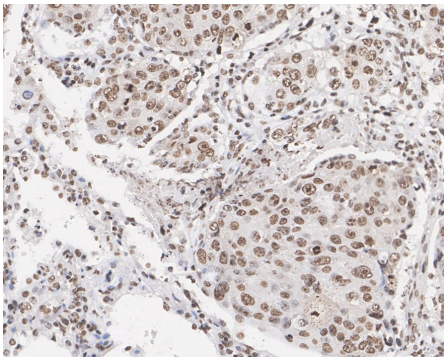

Fig3: Immunohistochemical analysis of paraffin-embedded human lung squamous cell carcinoma tissue with Rabbit anti-Phospho-NF-κB p65 (S529) antibody (ET1604-27) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (ET1604-27) 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.

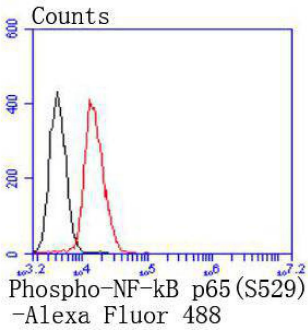

Fig4: Flow cytometric analysis of Phospho-NF-κB p65 (S529) was done on Daudi cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1604-27, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Yang K et al. Effect of PLCe gene silencing on inhibiting the cancerous transformation of ulcerative colitis. Exp Ther Med 12:422-426 (2016).
2. Jeon M et al. Elevated IL-1 expression induces invasiveness of triple negative breast cancer cells and is suppressed by zerumbone. Chem Biol Interact 258:126-33 (2016).