Data Sheet (Cat.No.TMAB-01469)

Anti-Phospho-NF-kB p65 (Thr435) Polyclonal Antibody

Product Details

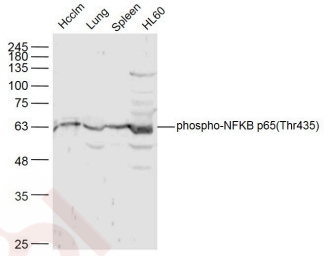
Ig Type:	IgG
Reactivity:	Human,Mouse (predicted:Rat)
Molecular Weight:	Theoretical: 61 kDa. Actual: 65 kDa.
Purification:	Protein A purified

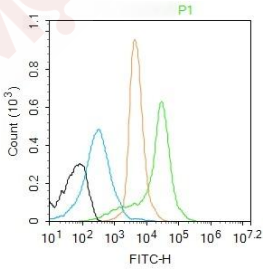
Applications

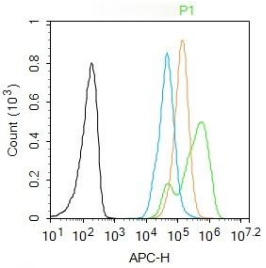
Verified Activity:

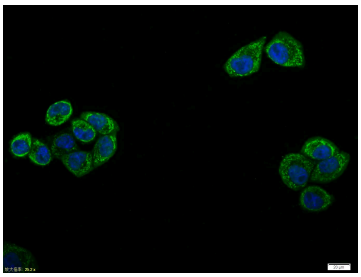
1. Sample:
Hcclm3 Cell (Human) Lysate at 40 µg
Lung (Mouse) Lysate at 40 µg
Spleen (Mouse) Lysate at 40 µg
HL60 Cell (Human) Lysate at 40 µg
Primary: Anti-phospho-NFKB p65 (Thr435) (TMAB-01469) at 1/300 dilution
Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution
Predicted band size: 61 kDa
Observed band size: 61 kDa
2. Blank control: Mouse spleen.
Primary Antibody (green line): Rabbit Anti-phospho-NFKB p65 (Thr435) antibody (TMAB-01469)
Dilution: 2 µg/10^6 cells;
Isotype Control Antibody (orange line): Rabbit IgG.
Secondary Antibody: Goat anti-rabbit IgG-AF488
Dilution: 1 µg/test.
Protocol
The cells were fixed with 4% PFA (10 min at room temperature) and then permeabilized with 90% ice-cold methanol for 20 min at-20°C. The cells were then incubated in 5% BSA to block non-specific protein-protein interactions for 30 min at room temperature. Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature.
3. Blank control: MCF7. Primary Antibody (green line): Rabbit Anti-phospho-NFKB p65 (Thr435) antibody (TMAB-01469)
Dilution: 2 µg/10^6 cells;
Isotype Control Antibody (orange line): Rabbit IgG.
Secondary Antibody: Goat anti-rabbit IgG-AF647
Dilution: 1 µg/test.
Protocol
The cells were fixed with 4% PFA (10 min at room temperature) and then permeabilized with 90% ice-cold methanol for 20 min at-20°C. The cells were then incubated in 5% BSA to block non-specific protein-protein interactions for 30 min at room temperature. Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature.
4. Tissue/cell: Hela cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum) at 37°C for 20 min; Antibody incubation with (phospho-NFKB p65 (Thr435)) polyclonal Antibody, Unconjugated (TMAB-01469) 1:100, 90 minutes at 37°C; followed by a FITC conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue) was used to stain the cell nucleus.

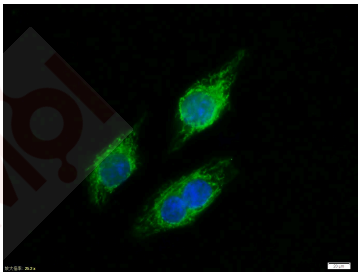
5. Tissue/cell: MCF7 cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum) at 37°C for 20 min; Antibody incubation with (phospho-NFKB p65 (Thr435)) polyclonal Antibody, Unconjugated (TMAB-01469) 1:100, 90 minutes at 37°C; followed by a FITC conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue) was used to stain the cell nucleus.
6. Tissue/cell: MCF7 cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum) at 37°C for 20 min; Antibody incubation with (phospho-NFKB p65 (Thr435)) polyclonal Antibody, Unconjugated (TMAB-01469) 1:100, 90 minutes at 37°C; followed by a FITC conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue) was used to stain the cell nucleus.
7. Blank control: A431. Primary Antibody (green line): Rabbit Anti-phospho-NFKB p65 (Thr435) antibody (TMAB-01469)
Dilution: 1 μg/10^6 cells;
Isotype Control Antibody (orange line): Rabbit IgG.
Secondary Antibody: Goat anti-rabbit IgG-FITC
Dilution: 1 μg/test.
Protocol
The cells were fixed with 4% PFA (10 min at room temperature) and then permeabilized with 90% ice-cold methanol for 20 min at-20°C. The cells were then incubated in 5% BSA to block non-specific protein-protein interactions for 30 min at room temperature. Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature.

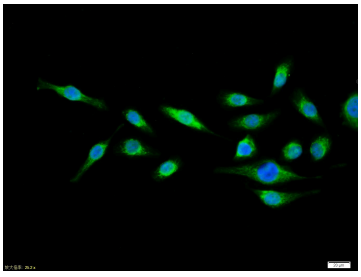


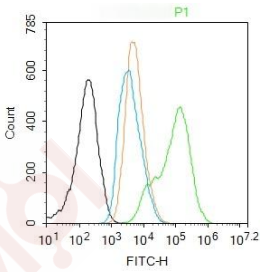












Application:	FCM,ICC/IF,WB
Recommended	FCM=2 μg/Test; ICC/IF=1:100-500; WB=1:500-2000

Properties

Stability & Storage:	Store at -20°C or -80°C for 12 months. Avoid repeated freeze-thaw cycles.
Shipping:	Shipping with blue ice.

Antigen Details

Immunogen:	KLH conjugated Synthesised phosphopeptide: human NFKBp65 around the phosphorylation site of Thr435
Antigen Species:	Human
Gene ID:	5970
Uniprot ID:	Q04206
Synonyms:	p-NF-kB p65 (Thr435);p-NF-kB p65 (T435);NF-kB p65 (p-Thr435);NF-kB p65 (p-T435);p65;v-rel avian reticuloendotheliosis viral oncogene homolog A;NFKB3
Biology Area:	RelA (p65),NFkB pathway,Inflammatory mediators,RelA (p65),NFkB pathway,Obesity,Host Immune Response,Host Virus Interaction,Alzheimer's disease,NFkB Pathway

Research Background

NF-kappa-B is a ubiquitous transcription factor involved in several biological processes. It is held in the cytoplasm in an inactive state by specific inhibitors. Upon degradation of the inhibitor, NF-kappa-B moves to the nucleus and activates transcription of specific genes. NF-kappa-B is composed of NFKB1 or NFKB2 bound to either REL, RELA, or RELB. The most abundant form of NF-kappa-B is NFKB1 complexed with the product of this gene, RELA. Four transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Sep 2011].

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