

## Anti-Phospho-IkB alpha/NFKBIA (Tyr305) Polyclonal Antibody

### Product Details

Ig Type:	IgG
Reactivity:	Human, Mouse, Rat (predicted: Pig, Cow, Rabbit, Sheep)
Molecular Weight:	Theoretical: 35 kDa. Actual: 38 kDa.
Purification:	Protein A purified

### Applications

1. Paraformaldehyde-fixed, paraffin embedded (mouse brain); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (phospho-IkB alpha (Tyr305)) Polyclonal Antibody, Unconjugated (TMAB-01428) at 1:400 overnight at 4°C, followed by a conjugated secondary antibody for 20 min and DAB staining.

2. Paraformaldehyde-fixed, paraffin embedded (Mouse liver); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (p-IkB alpha (Tyr305)) Polyclonal Antibody, Unconjugated (TMAB-01428) at 1:400 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

3. Tissue/cell: human colon carcinoma; 4% Paraformaldehyde-fixed and paraffin-embedded; Antigen retrieval: citrate buffer (0.01M, pH6.0), Boiling bathing for 15 min; Block endogenous peroxidase by 3% Hydrogen peroxide for 30 min; Blocking buffer (normal goat serum) at 37°C for 20 min;

Incubation: Anti-NFKBIA Polyclonal Antibody, Unconjugated (TMAB-01428) 1:200, overnight at 4°C, followed by conjugation to the secondary antibody and DAB staining.

#### Verified Activity:

4. Blank control (blue line): Jurkat (fixed with 2% paraformaldehyde (10 min), then permeabilized with 90% ice-cold methanol for 30 min on ice).

Primary Antibody (green line): Rabbit Anti-phospho-IkB alpha (Tyr305) antibody (TMAB-01428), Dilution: 1 µg/10<sup>6</sup> cells;

Isotype Control Antibody (orange line): Rabbit IgG.

Secondary Antibody (white blue line): Goat anti-rabbit IgG-FITC, Dilution: 1 µg/test.

5. Sample: 293T Cell (Human) Lysate at 40 µg

Primary: Anti-p-NFKBIA (Tyr305)(TMAB-01428) at 1/300 dilution

Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution

Predicted band size: 35 kDa

Observed band size: 38 kDa

6. Blank control (blue line): Hela (fixed with 70% methanol (Overnight at 4°C) and then permeabilized with 90% ice-cold methanol for 20 min at -20°C).

Primary Antibody (green line): Rabbit Anti-phospho-IkB alpha (Tyr305) antibody (TMAB-01428), Dilution: 1 µg/10<sup>6</sup> cells;

Isotype Control Antibody (orange line): Rabbit IgG.

Secondary Antibody (white blue line): Goat anti-rabbit IgG-PE, Dilution: 1 µg/test.

7. Blank control: Hela.

Primary Antibody (green line): Rabbit Anti-phospho-IkB alpha (Tyr305) antibody (TMAB-01428) Dilution: 1 µg/10<sup>6</sup> cells;

Isotype Control Antibody (orange line): Rabbit IgG.

Secondary Antibody: Goat anti-rabbit IgG-AF647

Dilution: 1 µg/test.

Protocol

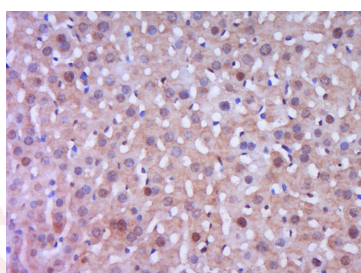
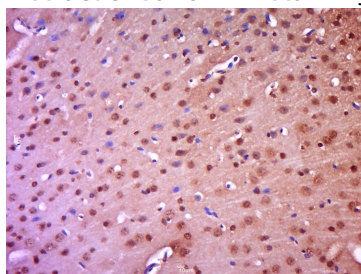
The cells were fixed with 4% PFA (10 min at room temperature) and then permeabilized with 90% ice-cold methanol for 20 min at -20°C. The cells were then incubated in 5% BSA to block non-specific protein-protein interactions for 30 min at room temperature. Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature.

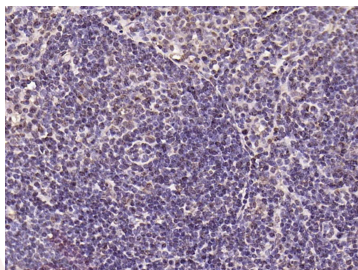
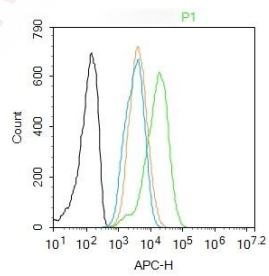
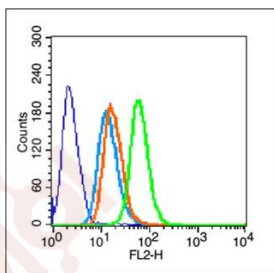
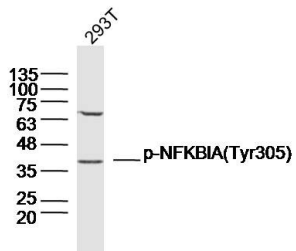
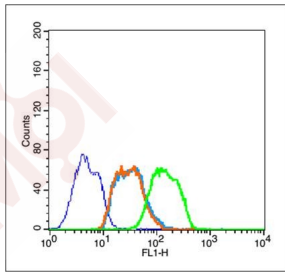
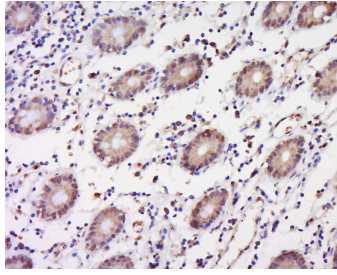
8. Paraformaldehyde-fixed, paraffin embedded (rat lymphoid); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (phospho-IKB alpha (Tyr305)) Polyclonal Antibody, Unconjugated (TMAB-01428) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

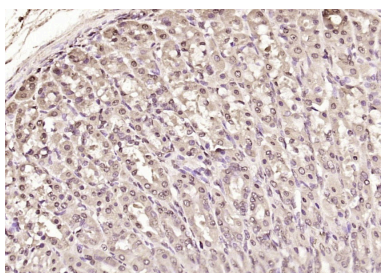
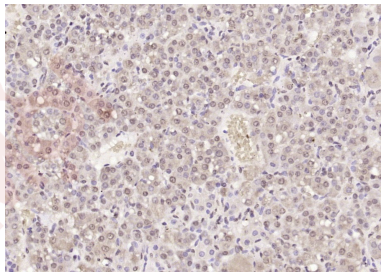
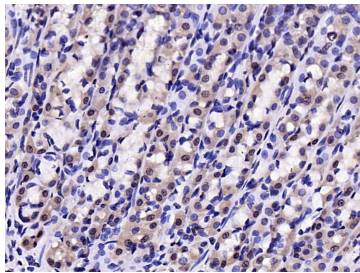
9. Paraformaldehyde-fixed, paraffin embedded (rat stomach); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (phospho-IKB alpha (Tyr305)) Polyclonal Antibody, Unconjugated (TMAB-01428) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

10. Paraformaldehyde-fixed, paraffin embedded (rat adrenal gland); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (phospho-IKB alpha (Tyr305)) Polyclonal Antibody, Unconjugated (TMAB-01428) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

11. Paraformaldehyde-fixed, paraffin embedded (rat stomach); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (phospho-IKB alpha (Tyr305)) Polyclonal Antibody, Unconjugated (TMAB-01428) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.







Application: FCM,IF,IHC-Fr,IHC-P,WB

Recommended WB: 1:500-2000; IHC-P: 1:100-500; IHC-Fr: 1:100-500; IF: 1:100-500; FCM: 1µg/Test

### 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 IκB alpha around the phosphorylation site of Tyr305

Antigen Species: Human

Gene ID: 4792

Uniprot ID: P25963

Synonyms: NFKBI;MAD-3;IκB α;p-IκB alpha/NFKBIA (Tyr305);IκB alpha/NFKBIA (p-Y305);nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, α;IκB alpha/NFKBIA (p-Tyr305);IKBA;nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha;p-IκB alpha/NFKBIA (Y305);nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor, alpha

Biology Area: NFκB pathway,NFκB pathway,TLR Signaling,NFκB Pathway

### Research Background

This gene encodes a member of the NF-kappa-B inhibitor family, which contain multiple ankrin repeat domains. The encoded protein interacts with REL dimers to inhibit NF-kappa-B/REL complexes which are involved in inflammatory responses. The encoded protein moves between the cytoplasm and the nucleus via a nuclear localization signal and CRM1-mediated nuclear export. Mutations in this gene have been found in ectodermal dysplasia anhidrotic with T-

cell immunodeficiency autosomal dominant disease. [provided by RefSeq, Aug 2011]

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