

## Anti-RNF25 Antibody Picoband®

Catalog Number: A10595-3

### About RNF25

E3 ubiquitin-protein ligase RNF25 is an enzyme that in humans is encoded by the RNF25 gene. The protein encoded by this gene contains a RING finger motif. The mouse counterpart of this protein has been shown to interact with RelA, the p65 subunit of NF-kappaB (NFKB), and modulate NFKB-mediated transcription activity. The mouse protein also binds ubiquitin-conjugating enzymes (E2s) and is a substrate for E2-dependent ubiquitination.

### Overview

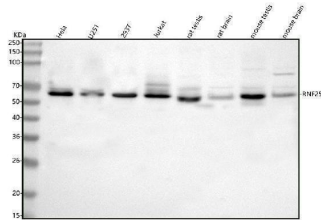
Product Name	Anti-RNF25 Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-RNF25 Antibody Picoband® catalog # A10595-3. Tested in WB, IHC, IF, FCM, ELISA applications. This antibody reacts with Human, Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	ELISA, Flow Cytometry, IF, IHC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl, 0.2 mg Na <sub>2</sub> HPO <sub>4</sub> .
Storage Instructions	At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.
Host	Rabbit
Uniprot ID	Q96BH1

### Technical Details

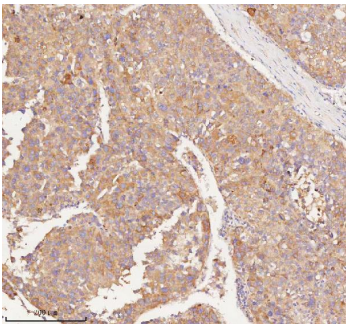
Immunogen	E.coli-derived human RNF25 recombinant protein (Position: E44-R344). Human RNF25 shares 83.7% amino acid (aa) sequence identity with mouse RNF25.
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P).
Cross Reactivity	No cross reactivity with other proteins.
Isotype	IgG
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.

Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.25-0.5 ug/ml, Human, Mouse, Rat Immunohistochemistry(Paraffin-embedded Section), 2-5 ug/ml, Human, Rat Immunofluorescence, 5 ug/ml, Human Flow Cytometry (Fixed), 1-3 ug/1x10 <sup>6</sup> cells, Human ELISA, 0.1-0.5 ug/ml, -

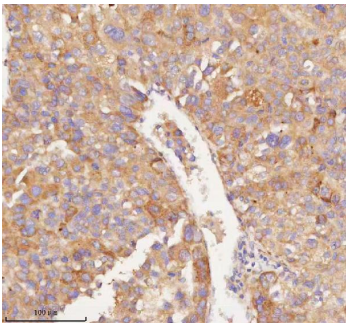
## Anti-RNF25 Antibody Picoband® (A10595-3) Images



Western blot analysis of RNF25 using anti-RNF25 antibody (A10595-3). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human HeLa whole cell lysates, Lane 2: human U251 whole cell lysates, Lane 3: human 293T whole cell lysates, Lane 4: human Jurkat whole cell lysates, Lane 5: rat testis tissue lysates, Lane 6: rat brain tissue lysates, Lane 7: mouse testis tissue lysates, Lane 8: mouse brain tissue lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-RNF25 antigen affinity purified polyclonal antibody (Catalog # A10595-3) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for RNF25 at approximately 62 kDa. The expected band size for RNF25 is at 51 kDa.

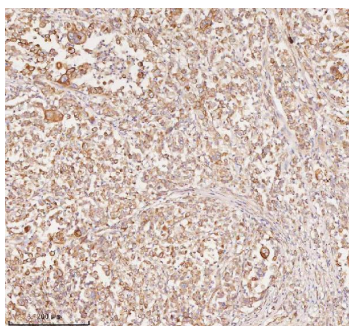


IHC analysis of RNF25 using anti-RNF25 antibody (A10595-3). RNF25 was detected in a paraffin-embedded section of human liver cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-RNF25 Antibody (A10595-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

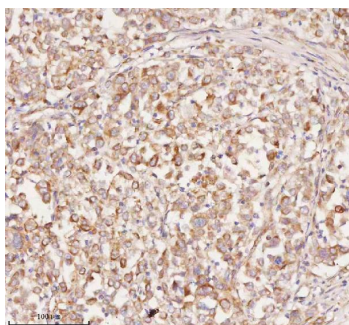


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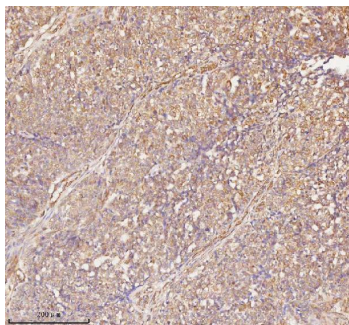
IHC analysis of RNF25 using anti-RNF25 antibody (A10595-3). RNF25 was detected in a paraffin-embedded



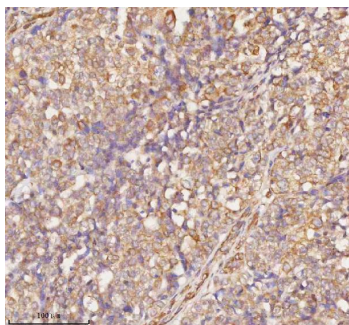
section of human lung adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-RNF25 Antibody (A10595-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.



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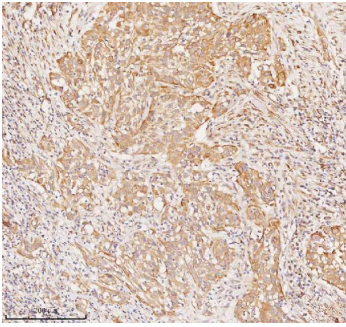


IHC analysis of RNF25 using anti-RNF25 antibody (A10595-3). RNF25 was detected in a paraffin-embedded section of human ovarian cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-RNF25 Antibody (A10595-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

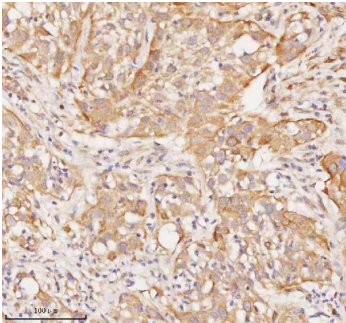


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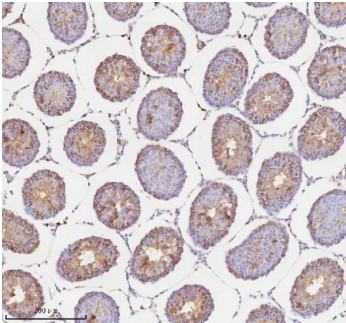
IHC analysis of RNF25 using anti-RNF25 antibody (A10595-3). RNF25 was detected in a paraffin-embedded section of human urothelial carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked



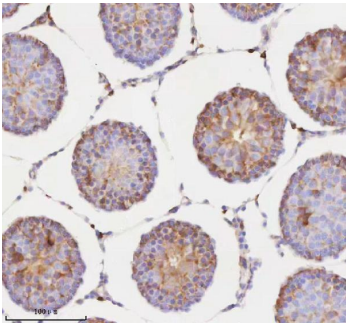
with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-RNF25 Antibody (A10595-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.



IHC analysis of RNF25 using anti-RNF25 antibody (A10595-3). RNF25 was detected in a paraffin-embedded section of human urothelial carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-RNF25 Antibody (A10595-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

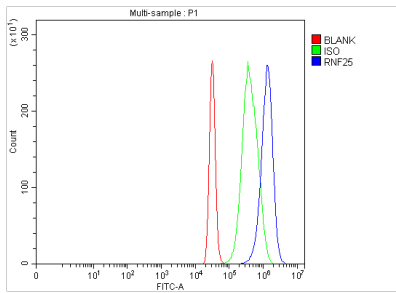


IHC analysis of RNF25 using anti-RNF25 antibody (A10595-3). RNF25 was detected in a paraffin-embedded section of rat testis tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-RNF25 Antibody (A10595-3) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

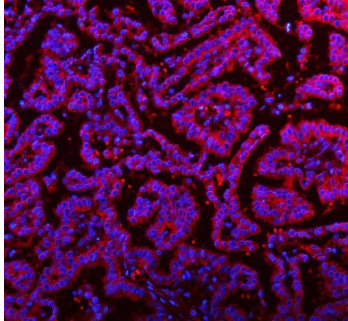


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Flow Cytometry analysis of 293T cells using anti-RNF25 antibody (A10595-3). Overlay histogram showing 293T cells stained with A10595-3 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were



blocked with 10% normal goat serum. And then incubated with rabbit anti-RNF25 Antibody (A10595-3, 1 ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.



IF analysis of RNF25 using anti-RNF25 antibody (A10595-3). RNF25 was detected in a paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 5 ug/mL rabbit anti-RNF25 Antibody (A10595-3) overnight at 4°C. DyLight®594 Conjugated Goat Anti-Rabbit IgG (BA1142) was used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

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### Anti-RNF25 Antibody

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