

## Product Datasheet

# TRIM25/EFP Rabbit Polyclonal Antibody (orb738470)

|                             |   |
|-----------------------------|---|
| <b>Catalog Number</b>       | orb738470   |
| <b>Category</b>             | Antibodies  |
| <b>Description</b>          | Anti-TRIM25/EFP Antibody. Tested in ELISA, Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Mouse, Rat. |
| <b>Target</b>               | E3 ubiquitin/ISG15 ligase TRIM25  |
| <b>Clonality</b>            | Polyclonal  |
| <b>Species/Host</b>         | Rabbit  |
| <b>Isotype</b>              | Rabbit IgG  |
| <b>Conjugation</b>          | Unconjugated  |
| <b>Reactivity</b>           | Mouse, Rat  |
| <b>Form/Appearance</b>      | Lyophilized   |
| <b>Concentration</b>        | 500 µg/ml   |
| <b>Buffer/Preservatives</b> | Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na <sub>2</sub> HPO <sub>4</sub> .                                       |
| <b>Reconstitution</b>       | Add 0.2ml of distilled water will yield a concentration of 500ug/ml.  |
| <b>Purification</b>         | Immunogen affinity purified.  |
| <b>Immunogen</b>            | E.coli-derived mouse TRIM25/EFP recombinant protein (Position: M1-H590).  |
| <b>UniProt ID</b>           | <b>Q61510</b>   |

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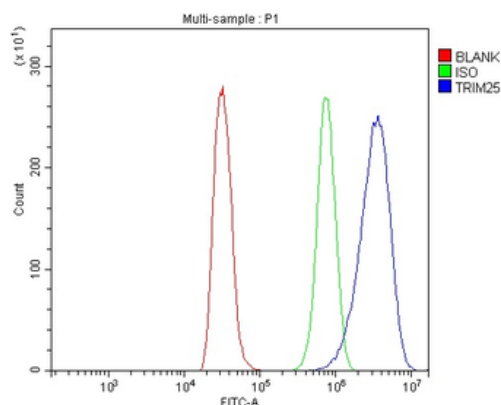
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|                            |   |
|----------------------------|---|
| <b>MW</b>                  | 71 kDa  |
| <b>Tested applications</b> | ELISA, FC, ICC, IF, IHC, WB   |
| <b>Dilution range</b>      | Western blot, 0.25-0.5µg/ml, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5µg/ml, Mouse, Rat Immunocytochemistry/Immunofluorescence, 5µg/ml, Mouse Flow Cytometry (Fixed), 1-3µg/1x10 <sup>6</sup> cells, Mouse, Rat ELISA, 0.1-0.5µg/ml, - |
| <b>Cross Reactivity</b>    | No cross-reactivity with other proteins.  |
| <b>Antibody Type</b>       | Primary Antibody  |
| <b>Storage</b>             | Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.   |
| <b>Note</b>                | For research use only   |
| <b>Expiration Date</b>     | 12 months from date of receipt.   |



Flow Cytometry analysis of NRK cells using anti-TRIM25/EFP antibody. Overlay histogram showing NRK cells (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-TRIM25/EFP Antibody (1 µg/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 µg/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 µg/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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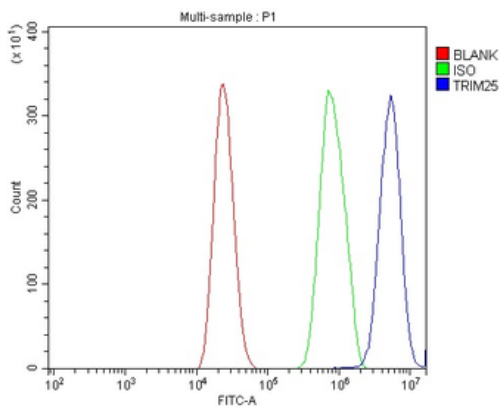
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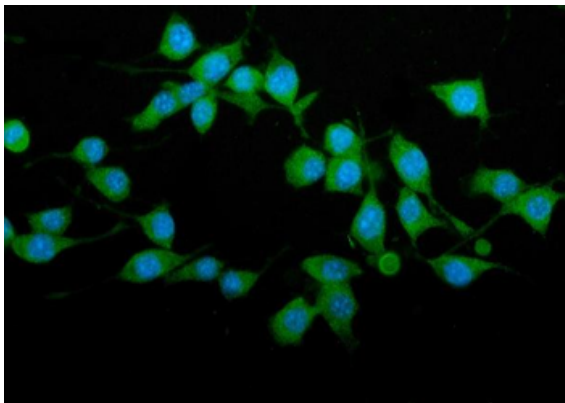
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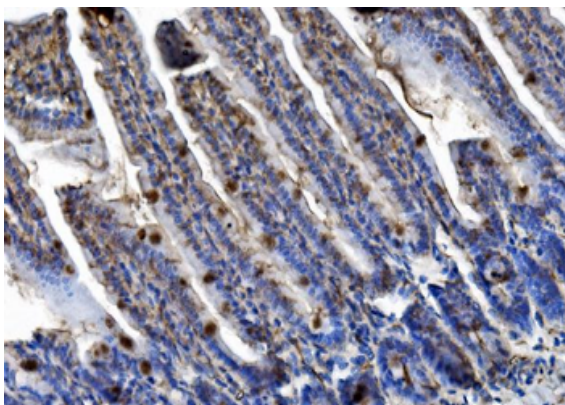
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Flow Cytometry analysis of RAW264.7 cells using anti-TRIM25/EFP antibody. Overlay histogram showing RAW264.7 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-TRIM25/EFP Antibody (1 µg/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 µg/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 µg/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



IF analysis of TRIM25/EFP using anti-TRIM25/EFP antibody. TRIM25/EFP was detected in immunocytochemical section of MFC cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL rabbit anti-TRIM25/EFP Antibody overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:100 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.



IHC analysis of TRIM25/EFP using anti-TRIM25/EFP antibody. TRIM25/EFP was detected in paraffin-embedded section of mouse intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-TRIM25/EFP Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.

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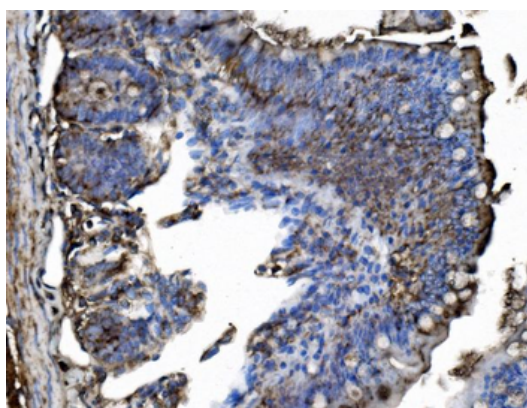
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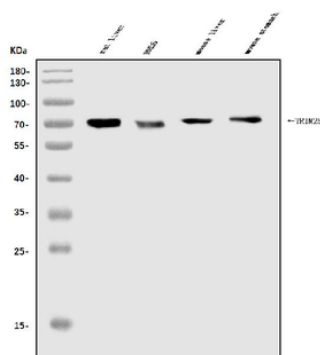
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IHC analysis of TRIM25/EFP using anti-TRIM25/EFP antibody. TRIM25/EFP was detected in paraffin-embedded section of rat intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-TRIM25/EFP Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.



Western blot analysis of TRIM25/EFP using anti-TRIM25/EFP antibody. 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 µg of sample under reducing conditions. Lane 1: rat liver tissue lysates, Lane 2: rat RH35 whole cell lysates, Lane 3: mouse liver tissue lysates, Lane 4: mouse stomach 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-TRIM25/EFP antigen affinity purified polyclonal antibody at 0.5 µg/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 with Tanon 5200 system. A specific band was detected for TRIM25/EFP at approximately 71 KD. The expected band size for TRIM25/EFP is at 71 KD.

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