Anti-IL3RA / CD123 Antibody

HA500291



Product Type: Rabbit polyclonal IgG, primary antibodies

Species reactivity: Human

Applications: WB, IF-Cell, IHC-P, FC

Molecular Wt: Predicted band size: 43 kDa

Description: The protein encoded by this gene is an interleukin 3 specific subunit of a heterodimeric

cytokine receptor. The receptor is comprised of a ligand specific alpha subunit and a signal transducing beta subunit shared by the receptors for interleukin 3 (IL3), colony stimulating factor 2 (CSF2/GM-CSF), and interleukin 5 (IL5). The binding of this protein to IL3 depends on the beta subunit. The beta subunit is activated by the ligand binding, and is required for the biological activities of IL3. This gene and the gene encoding the colony stimulating factor 2 receptor alpha chain (CSF2RA) form a cytokine receptor gene cluster in a X-Y pseudoautosomal region on chromosomes X or Y. Alternatively spliced transcript variants

encoding distinct isoforms have been found.

Immunogen: Synthetic peptide within human IL3RA aa 231-280.

Positive control: MCF-7 cell lysates, Hela, human fallopian tube tissue, A549.

Subcellular location: Membrane.

Database links: SwissProt: P26951 Human

Recommended Dilutions:

WB 1:500 IF-Cell 1:200 IHC-P 1:600

FC 1:500-1:1,000

Storage Buffer: PBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4° C. Store at $+4^{\circ}$ C short term (1-2 weeks). It is recommended to aliquot into

single-use upon delivery. Store at -20 ℃ long term.

Purity: Immunogen affinity purified.

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Images

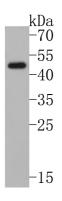


Fig1: Western blot analysis of IL3RA / CD123 on MCF-7 cell lysates. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA500291, 1/500) was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

Predicted band size: 43 kDa Observed band size: 45 kDa

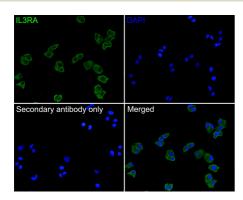


Fig2: ICC staining of IL3RA / CD123 in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (HA500291, 1/200) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

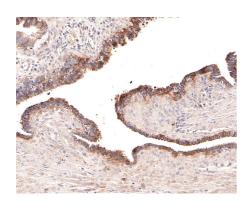


Fig3: Immunohistochemical analysis of paraffin-embedded human fallopian tube tissue using anti-IL3RA / CD123 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA500291, 1/600) for 30 minutes 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.

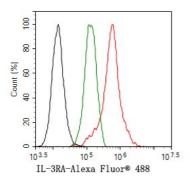


Fig4: Flow cytometric analysis of A549 cells labeling IL3RA / CD123.

Cells were washed twice with cold PBS and resuspend. Then stained with the primary antibody (HA500291, 1ug/ml) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4 $^{\circ}$ C for an hour, the cells were stained with a Alexa Fluor® 488 conjugate-Goat anti-Rabbit IgG Secondary antibody at 1/1,000 dilution for 30 minutes at +4 $^{\circ}$ C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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

Background References

- 1. Kirchhoff D. et. al. IL3RA-Targeting Antibody-Drug Conjugate BAY-943 with a Kinesin Spindle Protein Inhibitor Payload Shows Efficacy in Preclinical Models of Hematologic Malignancies. Cancers (Basel). 2020 Nov
- 2. Agger K. et. al. Jmjd2/Kdm4 demethylases are required for expression of II3ra and survival of acute myeloid leukemia cells. Genes Dev. 2016 Jun