# Anti-Rab5 Antibody [ST47-04]

# ET1609-27



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell
Molecular Wt:	Predicted band size: 24 kDa
Clone number:	ST47-04
Description:	The Ras-related superfamily of guanine nucleotide binding proteins, which includes the R-Ras, Rap, Ral/Rec and Rho/Rab subfamilies, exhibit 30-60% homology with Ras p21. Accumulating data suggests an important role for Rab proteins, either in endocytosis or in biosynthetic protein transport. The transport of newly synthesized proteins from the endoplasmic reticulum to various stacks of the Golgi complex and to secretory vesicles involves at each stage the movement of carrier vesicles, a process that appears to involve Rab protein function. The possibility that Rab proteins might also direct the exocytosis from secretory vesicles to the plasma membrane is supported by the observation that in yeast, the Sec4 protein, which is 40% homologous to Rab proteins, is associated with secretory vesicles. At least eight members of the Rab subfamily have been identified, each of which is found at a particular stage of a membrane transport pathway.
Immunogen:	Synthetic peptide within Human Rab5 aa 166-215 / 215.
Positive control:	MCF-7 cell lysate, Hela cell lysate, Mouse brain tissue lysate, Rat brain tissue lysate, Neuro-2a cell lysate, PC-12 cell lysate, human colon cancer tissue, human prostate cancer tissue, PC-12.
Subcellular location:	Cell membrane, Cytoplasm, Cell projection, Membrane, Endosome.
Database links:	SwissProt: P20339 Human   Q9CQD1 Mouse   M0RC99 Rat
Recommended Dilutions: WB IHC-P IF-Cell	1:500-1:2,000 1:50-1:200 1:100
Storage Buffer:	1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Store at +4 $^\circ\!C$ after thawing. Aliquot store at -20 $^\circ\!C$ or -80 $^\circ\!C$ . Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

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#### Images



**Fig1:** Western blot analysis of Rab5 on different lysates with Rabbit anti-Rab5 antibody (ET1609-27) at 1/2,000 dilution.

Lane 1: HAP1-parental cell lysate Lane 2: HAP1-Rab5 KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 24 kDa Observed band size: 24 kDa

Exposure time: 2 minutes; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1609-27) at 1/2,000 dilution was used in primary antibody dilution (K1803) at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

**Fig2:** Western blot analysis of Rab5 on different lysates with Rabbit anti-Rab5 antibody (ET1609-27) at 1/1,000 dilution.

Lane 1: MCF-7 cell lysate Lane 2: Hela cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 24 kDa Observed band size: 24 kDa

Exposure time: 1 minute;

12% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1609-27) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.



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**Fig3:** Western blot analysis of Rab5 on different lysates with Rabbit anti-Rab5 antibody (ET1609-27) at 1/1,000 dilution.

Lane 1: Mouse brain tissue lysate Lane 2: Rat brain tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 24 kDa Observed band size: 24 kDa

Exposure time: 2 minutes;

12% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1609-27) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

**Fig4:** Western blot analysis of Rab5 on different lysates with Rabbit anti-Rab5 antibody (ET1609-27) at 1/1,000 dilution.

Lane 1: Neuro-2a cell lysate Lane 2: PC-12 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 24 kDa Observed band size: 24 kDa

Exposure time: 3 minutes; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1609-27) at 1/1,000 dilution was used in 5% NFDM/TBST at  $4^{\circ}$ C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.



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kDa

75

35

25

14

Rab5

- 🕳 GAPDH

~24kDa

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**Fig5:** Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-Rab5 antibody (ET1609-27) at 1/200 dilution.

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 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1609-27) at 1/200 dilution for 1 hour 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.



**Fig6:** Immunohistochemical analysis of paraffin-embedded human prostate cancer tissue with Rabbit anti-Rab5 antibody (ET1609-27) at 1/200 dilution.

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 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1609-27) at 1/200 dilution for 1 hour 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.

**Fig7:** Immunocytochemistry analysis of PC-12 cells labeling Rab5 with Rabbit anti-Rab5 antibody (ET1609-27) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Rab5 antibody (ET1609-27) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at  $+4^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor  $\pm$  594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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

#### **Background References**

- 1. Suo D et al. Coronin-1 is a neurotrophin endosomal effector that is required for developmental competition for survival. Nat Neurosci 17:36-45 (2014).
- 2. Simon NC & Barbieri JT Exoenzyme S ADP-ribosylates Rab5 effector sites to uncouple intracellular trafficking. Infect Immun 82:21-8 (2014).

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

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