# Endosomal Marker Antibody Sampler Kit HAK21027

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
Caveolin-1 [ET1603-1]	20μ1	WB, IF-Cell, IF-Tissue, IHC-P	H, M, R	20 kDa
Clathrin heavy chain [ET1704-50]	20μ1	WB,IHC-P	H,M,R,Mk	180kDa
EEA1 [HA722147]	20μ1	WB, IF-Cell, FC	H, M, R, Mk	162 kDa
Rab5 [ET1609-27]	20μ1	WB,IHC-P,IF-Cell	H, M, R	24 kDa
RAB7 [ET1611-96]	20μ1	WB, IF-Cell, IF-Tissue, IHC-P, FC	H, M, R	23 kDa
Rab11A [HA721552]	20μ1	WB,IHC-P	H, M, R	24 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100μ1	WB, ELISA, IHC-P	Rab	

#### Description:

Endosomal Marker Antibody Sampler Kit provides an economical means of distinguishing endosomes in the early, late, and recycling phases. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

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. Avoid repeated freeze / thaw cycles.

# Background

Endosomes are formed by the invagination of the plasma membrane to form vesicles in an effort to recycle components of the cell. Endosomes can be coated in clathrin when vesicles form at clathrin-coated pits.

Caveolins are 21-24 kDa integral proteins that interact with cholesterol and are the main structural components of the cholesterol/sphingolipid-enriched plasma membrane caveolae.

Each stage of endosome maturation is marked by a unique set of proteins. EEA1 is an early endosome marker that is essential for membrane fusion and trafficking. Members of the ras superfamily of small Rab GTPases, specifically Rab5, Rab7, and Rab11 are markers of the early, late and recycling endosomes.

#### Database links:

UniProt ID: Q03135, P49817, P41350, Q00610, Q68FD5, P11442, Q15075, Q8BL66, 314764, P20339, Q9CQD1, M0RC99, P51149, P51150, P09527, P62491, P62492, P62494

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## **Images**

Clathrin heavy chain 1 -180kDa 100 72 55 42 35 25

Fig1: Western blot analysis of Clathrin heavy chain on different lysates with Rabbit anti-Clathrin heavy chain antibody (ET1704-50) at 1/1,000 dilution.

Lane 1: HeLa cell lysate Lane 2: A431 cell lysate Lane 3: NIH/3T3 cell lysate Lane 4: PC-12 cell lysate Lane 5: HUVEC cell lysate Lane 6: SH-SY5Y cell lysate Lane 7: COS-1 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 180kDa Observed band size: 180 kDa

Exposure time: 10 seconds;

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 (ET1704-50) at 1/1,000 dilution was used in 5% NFDM/TBST 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.

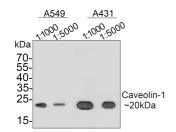
Fig2: Western blot analysis of Caveolin-1 on different lysates with Rabbit anti-Caveolin-1 antibody (ET1603-1) at different dilutions.

Lane 1/2: A549 cell lysate Lane 3/4: A431 cell lysate

Lysates/proteins at 10 µg/Lane. Predicted band size: 20 kDa Observed band size: 20 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 (ET1603-1) at different dilutions were 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:** All lanes: Western blot analysis of RAB7 with anti-RAB7 antibody (ET1611-96) at 1:500 dilution.

Lane 1: Wild-type Hela whole cell lysate (10 µg).

Lane 2/3: RAB7 knockdown Hela whole cell lysate (10 µg).

ET1611-96 was shown to specifically react with RAB7 in wild-type Hela cells. Weakened bands were observed when RAB7 knockdown samples were tested. Wild-type and RAB7 knockdown samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1611-96, 1:500) was used in 5% BSA 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 RAB7 on different lysates with Rabbit anti-RAB7 antibody (ET1611-96) at 1/5,000 dilution.

Lane 1: HeLa cell lysate Lane 2: MCF7 cell lysate Lane 3: Neuro-2a cell lysate Lane 4: C2C12 cell lysate Lane 5: PC-12 cell lysate Lane 6: C6 cell lysate

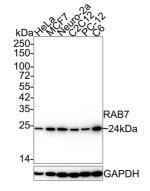
Lysates/proteins at 15 µg/Lane.

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

Exposure time: 17 seconds;

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 (ET1611-96) at 1/5,000 dilution was used in 5% NFDM/TBST at  $4^{\circ}\mathrm{C}$  overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.



kDax 250-150-150-75-50-37-25-20-15-10-GAPDH Fig5: Western blot analysis of Rab11A on different lysates with Rabbit anti-Rab11A antibody (HA721552) at 1/1,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)

Lane 2: A549 cell lysate (20 µg/Lane)

Lane 3: SH-SY5Y cell lysate (20 µg/Lane)

Lane 4: Neuro-2a cell lysate (20 µg/Lane)

Lane 5: C6 cell lysate (20 µg/Lane)

Lane 6: Mouse testis tissue lysate (40 µg/Lane)

Lane 7: Rat testis tissue lysate (40 µg/Lane)

Lane 8: Rat brain tissue lysate (40 µg/Lane)

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

Exposure time: 2 minutes 37 seconds;

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 (HA721552) 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 1:100,000 dilution was used for 1 hour at room temperature.

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

## **Background References**

- 1. Huotari, J. and Helenius, A. (2011) EMBO J 30, 3481-500.
- 2. Rodriguez-Boulan, E. et al. (2005) Nat Rev Mol Cell Biol 6, 233-47.
- 3. Okamoto, T. et al. (1998) J Biol Chem 273, 5419-22.
- 4. Christoforidis, S. et al. (1999) Nature 397, 621-5.
- 5. Zerial, M. and McBride, H. (2001) Nat Rev Mol Cell Biol 2, 107-17.

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