Vesicle Trafficking Antibody Sampler Kit

#9765 Store at -20°C

1 Kit
(8 x 20 µl)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-Caveolin-1 (Tyr14) Antibody	3251	20 µl	23, 25 kDa	Rabbit IgG
Caveolin-1 (D46G3) XP™ Rabbit mAb	3267	20 µl	21, 24 kDa	Rabbit IgG
Clathrin Heavy Chain (D3C6) XP™ Rabbit mAb	4796	20 µl	190 kDa	Rabbit IgG
APPL1 (D83H4) XP™ Rabbit mAb	3858	20 µl	82 kDa	Rabbit IgG
EEA1 (C45B10) Rabbit mAb	3288	20 µl	170 kDa	Rabbit IgG
Syntaxin 6 (C34B2) Rabbit mAb	2869	20 µl	32 kDa	Rabbit IgG
GOPC (D10A12) Rabbit mAb	8576	20 µl	59 kDa	Rabbit IgG
Rab5A (E6N8S) Mouse mAb	46449	20 µl	25 kDa	Mouse IgG1
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The Vesicle Trafficking Antibody Sampler kit provides an economical means to analyze proteins involved in the intracellular transport of cargo proteins. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Vesicle trafficking is an integral cellular process and the associated proteins involved also play major roles in other signaling pathways. Caveolins are involved in diverse biological functions including vesicular trafficking, cholesterol homeostasis, cell adhesion, apoptosis, and are also indicated in neurodegenerative disease (1). It is believed that caveolins serve as scaffolding proteins for the integration of signal transduction. Phosphorylation at Tyr14 is essential for caveolin association with SH2 or PTB domain-containing adaptor proteins, such as GRB7 (2-4).

Clathrin-coated vesicles provide for the intracellular transport of proteins following endocytosis and during multiple vesicle trafficking pathways. Vesicles form at specialized areas of the cell membrane where clathrin and associated proteins form clathrin-coated pits. Invagination of these cell membrane-associated pits internalizes proteins and forms an intracellular clathrin-coated vesicle (5,6). Clathrin is the most abundant protein in these vesicles and is present as a basic assembly unit called a triskelion. Each clathrin triskelion is composed of three clathrin heavy chains and three clathrin light chains. Clathrin heavy chain proteins are composed of several functional domains that associate with other vesicle proteins (6).

The APPL1 multidomain adaptor protein is a BAR-domain protein family member that is involved in membrane trafficking within a number of signal transduction pathways (7).

EEA1 is an early endosomal marker and a Rab5 effector protein essential for early endosomal membrane fusion and trafficking (8,9). Syntaxin 6 is a ubiquitously expressed S25C family member of the SNARE proteins (10,11). Syntaxin 6 protein is localized to the trans-Golgi and within endosomes and regulates membrane trafficking by partnering with a variety of other SNARE proteins (12-14). It has two coiled-coil domains (CC1 and CC2) located in the amino-terminal region and a PDZ domain in the carboxy-terminal region (15). The CC2 domain and its adjacent linker region mediate the association of GOPC with the Golgi protein golgin-160 and the Q-SNARE protein syntaxin 6 (15,16). The PDZ domain of GOPC interacts with the carboxy terminus of target proteins to mediate target protein vesicular trafficking and surface expression (17-20).

Rab5 is a member of the Ras superfamily of small Rab GTPases. Rab5 is localized at the plasma membrane and early endosomes and functions as a key regulator of vesicular trafficking during early endocytosis (21).

Specificity/Sensitivity: Phospho-Caveolin-1 (Tyr14) Antibody detects endogenous levels of Caveolin-1 only when phosphorylated at Tyr14 and does not cross-react with caveolin-2, -3 or caveolin-1β, the short isoform of caveolin-1. Caveolin-1 (D46G3) XP® Rabbit mAb detects endogenous levels of total caveolin-1 protein. Clathrin Heavy Chain (D3C6) XP® Rabbit mAb detects endogenous levels of total clathrin protein. APPL1 (D83H4) XP® Rabbit mAb detects endogenous levels of total APPL1 protein. EEA1 (C45B10) Rabbit mAb detects endogenous levels of total EEA1 protein. Syntaxin 6 (C34B2) Rabbit mAb detects endogenous levels of total Syntaxin 6 protein. GOPC (D10A12) Rabbit mAb detects endogenous levels of total GOPC protein. Rab5A (E6N8S) Mouse mAb detects endogenous levels of total Rab5A protein.

Storage: All antibodies in this kit are supplied in 10mM HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:

Western blotting	1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Background References:

(1) Smart, E.J. et al. (1999) *Mol Cell Biol* 19, 7289-304.
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(4) Lee, H. et al. (2000) *Mol Endocrinol* 14, 1750-75.
(5) Rodriguez-Boulan, E. et al. (2005) *Nat Rev Mol Cell Biol* 6, 233-47.
(6) Mousavi, S.A. et al. (2004) *Biochem J* 377, 1-16.
(7) Habermann, B. (2004) *EMBO Rep* 5, 250-5.
(8) Mu, F.T. et al. (1995) *J Biol Chem* 270, 13503-11.
(9) Christoforidis, S. et al. (1999) *Nature* 397, 621-5.
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(11) Bock, J.B. et al. (1996) *J Biol Chem* 271, 17961-5.
(12) Wendler, F. and Tooze, S. (2001) *Traffic* 2, 606-11.
(13) Bock, J.B. et al. (1997) *Mol Biol Cell* 8, 1261-71.
(14) Mallard, F. et al. (2002) *J Cell Biol* 156, 653-64.
(15) Charest, A. et al. (2001) *J Biol Chem* 276, 29456-65.
(16) Hicks, S.W. and Machamer, C.E. (2005) *J Biol Chem* 280, 28944-51.
(17) Cheng, J. et al. (2002) *J Biol Chem* 277, 3520-9.
(18) He, J. et al. (2004) *J Biol Chem* 279, 50190-6.
(19) Wente, W. et al. (2005) *J Biol Chem* 280, 32419-25.
(20) Ito, H. et al. (2006) *Biochem J* 397, 389-98.
(21) Zerial, M. and McBride, H. (2001) *Nat Rev Mol Cell Biol* 2, 107-17.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Tyr14 of human caveolin-1. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Glu20 of human caveolin-1, Pro1663 of human clathrin heavy chain protein, Thr426 of human APPL1, Ser70 of human EEA1 protein, Tyr140 of mouse syntaxin 6 protein, Phe31 of human GOPC protein, and Gly190 of human Rab5A protein.

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Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#13953)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#13953, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.