#12666 Store at -20°C

Endosomal Marker Antibody Sampler Kit

☑ 1 Kit
(6 x 40 µl)

Cell Signaling TECHNOLOGY®

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rev. 03/27/14

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Caveolin-1 (D46G3) XP® Rabbit mAb	3267	40 µl	21, 24 kDa	Rabbit IgG
Clathrin Heavy Chain (D3C6) XP® Rabbit mAb	4796	40 µl	190 kDa	Rabbit IgG
EEA1 (C45B10) Rabbit mAb	3288	40 µl	170 kDa	Rabbit IgG
Rab5 (C8B1) Rabbit mAb	3547	40 µl	25 kDa	Rabbit IgG
Rab7 (D95F2) XP® Rabbit mAb	9367	40 µl	23 kDa	Rabbit IgG
Rab11 (D4F5) XP® Rabbit mAb	5589	40 µl	25 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

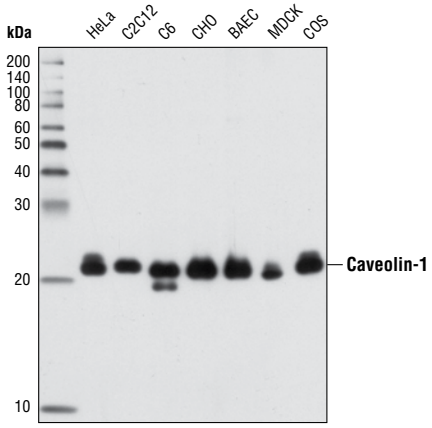
See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The 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 four western blot experiments with each primary antibody.

Background: Endosomes are formed by the invagination of the plasma membrane to form vesicles in an effort to recycle components of the cell (1). Endosomes can be coated in clathrin when vesicles form at clathrin-coated pits (2). 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 (3). 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 (4). Members of the ras superfamily of small Rab GTPases, specifically Rab5, Rab7, and Rab11 are markers of the early, late and recycling endosomes (5).

Specificity/Sensitivity: Each antibody will detect endogenous total levels of their target protein. The antibodies do not cross-react with other isoforms, with the exception of the Rab11 (D4F5) XP® Rabbit mAb which will detect both Rab11a and Rab11b.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Glu20 of human caveolin-1, Pro1663 of human clathrin heavy chain protein, Ser70 of human EEA1 protein, Gly190 of human Rab5A protein, Glu188 of human Rab7 protein, and near the amino terminus of human Rab11 protein.



Western blot analysis of extracts from various cell types using ***Caveolin-1 (D46G3) XP® Rabbit mAb #3267****.*

Storage: Supplied in 10 mM sodium 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 a complete listing of recommended companion products.

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.

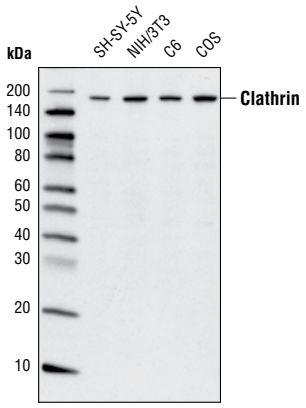
(3) Okamoto, T. et al. (1998) *J Biol Chem* 273, 5419-22.

(4) Christoforidis, S. et al. (1999) *Nature* 397, 621-5.

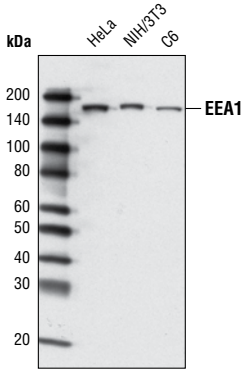
(5) Zerial, M. and McBride, H. (2001) *Nat Rev Mol Cell Biol* 2, 107-17.

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

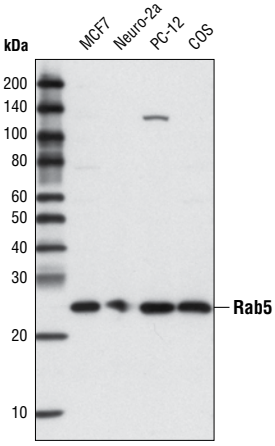
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted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



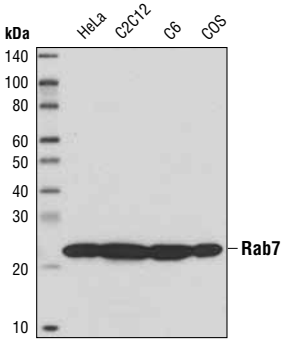
Western blot analysis of extracts from various cell lines using ***Clathrin Heavy Chain (D3C6) XP® Rabbit mAb #4796***.



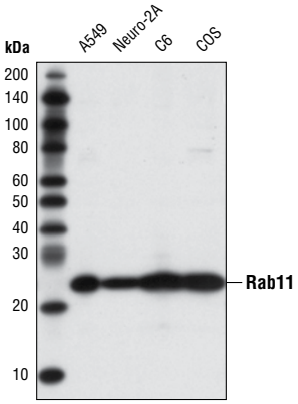
Western blot analysis of extracts from various cell lines using ***EEA1 (C45B10) Rabbit mAb #3288***.



Western blot analysis of extracts from various cell lines using ***Rab5 (C8B1) Rabbit mAb #3547***.



Western blot analysis of extracts from various cell lines using ***Rab7 (D95F2) XP® Rabbit mAb #9367***.



Western blot analysis of extracts from various cell lines using ***Rab11 (D4F5) XP® Rabbit mAb #5589***.

#12666

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 budder. 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):** (#7720)
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 (#7720, 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.