#12460 Store at -20°C

Eps15 (D3K8R) Rabbit mAb

☑ 100 µl
(10 western blots)

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New 02/13

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

Entrez-Gene ID #2060
Swiss-Prot Acc. #P42566

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC	H, M, R, Mk,	138 kDa	Rabbit IgG**
Endogenous	(C, X, Dg, Hr)		

Background: Eps15 (EGFR pathway substrate 15) was originally discovered as a substrate for the kinase activity of EGFR (1). Eps15 has a tripartite structure comprising an amino terminal portion, which contains three evolutionarily conserved EH protein-protein interaction domains, a central putative coiled-coil region required for constitutive oligmerization, and a carboxy terminal domain containing multiple copies of the amino acid triplet Asp-Pro-Phe that constitute the AP2 binding domain. The carboxy terminal domain also contains two ubiquitin interaction motifs (UIMs), the last of which is indespensible for Eps15 binding to ubiquitin (1). Several lines of evidence support a role for Eps15 in clathrin-mediated endocytosis, including the endocytosis of synaptic vesicles. Eps15 binds to AP2 as well as other proteins involved in endocytosis and/or synaptic vesicle recycling, such as synaptojanin1 and epsin. Furthermore, Eps15 colocalizes with markers of the plasma membrane clathrin-coated pits and vesicles (2). Eps15 regulates the endosomal trafficking of c-Met (3) and EGFR (4), possibly by recruiting the ubiquitinated receptors to the rims of clathrin-coated pits through interaction between the ubiquitin tag and its UIMs.

The *EPS15* gene yields two isoforms that are believed to reside in distinct subcellular locations and are thus implicated in different facets of endosomal trafficking (5). Human *EPS15* has been mapped to chromosome 1p31-p32, a region displaying several nonrandom chromosomal abnormalities, including deletions in neuroblastoma and translocation in acute lymphoblastic and myeloid leukemias. Research has shown two translocations t(1;11)(p32;q11) are found in rare cases of myeloid leukemia where the Eps15 gene was fused to the HRX gene, resulting in two reciprocal fusion genes (6).

Specificity/Sensitivity: Eps15 (D3K8R) Rabbit mAb recognizes endogenous levels of total Eps15 protein. Based upon sequence alignment, this antibody is predicted to react with both Eps15a and Eps15b. This antibody does not cross-react with Eps15R.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Pro630 of human Eps15 protein.

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 antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

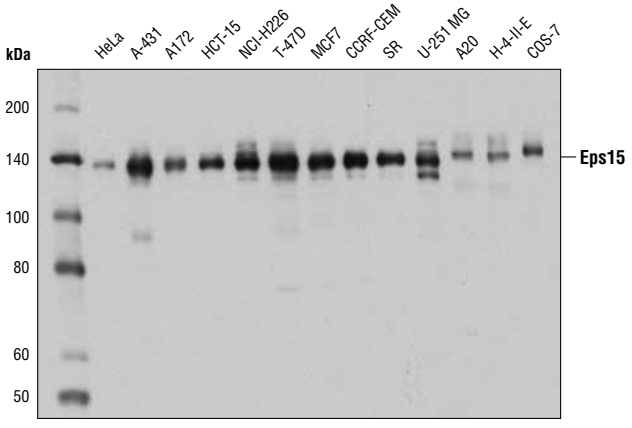
Western blotting	1:1000
Immunoprecipitation	1:100
Immunofluorescence (IF-IC)	1:200

For product specific protocols please see the web page for this product at www.cellsignal.com.

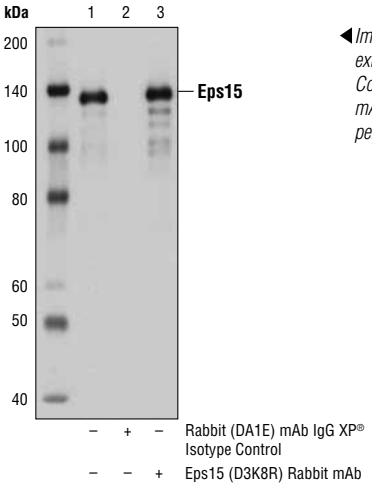
Please visit www.cellsignal.com for a complete listing of recommended complementary products.

Background References:

(1) Fazioli, F. et al. (1993) *Mol Cell Biol* 13, 5814-28.
(2) Tebar, F. et al. (1996) *J Biol Chem* 271, 28727-30.
(3) Parachoniak, C.A. and Park, M. (2009) *J Biol Chem* 284, 8382-94.
(4) Torrisi, M.R. et al. (1999) *Mol Biol Cell* 10, 417-34.
(5) Roxrud, I. et al. (2008) *J Cell Biol* 180, 1205-18.
(6) Bernard, O.A. et al. (1994) *Oncogene* 9, 1039-45.



Western blot analysis of extracts from various cell lines using Eps15 (D3K8R) Rabbit mAb.



◀ *Immunoprecipitation of Eps15 from MCF7 cell extracts using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (lane 2) or Eps15 (D3K8R) Rabbit mAb (lane 3). Lane 1 is 10% input. Western blot was performed using Eps15 (D3K8R) Rabbit mAb.*

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

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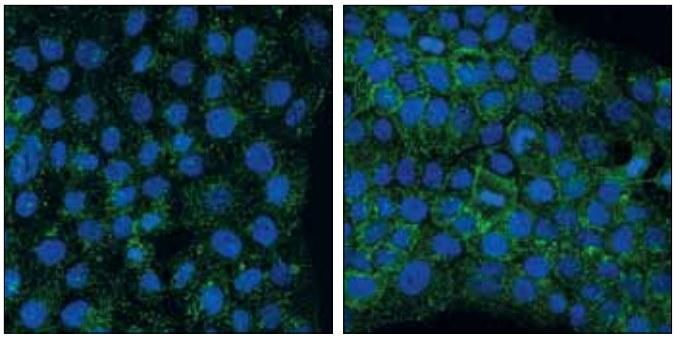
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Serum-starved **hEGF-treated**



Confocal immunofluorescent analysis of A-431 cells, serum-starved (left) or treated with Human Epidermal Growth Factor (hEGF) #8916 (100 ng/mL, 1 hr, 4ºC; right), using Eps15 (D3K8R) Rabbit mAb (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).