

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Eps15 in direct ELISAs and Western blots.
Source	Recombinant Monoclonal Rabbit IgG Clone # 1261C
Purification	Protein A or G purified from cell culture supernatant
Immunogen	<i>E. coli</i> -derived recombinant human Eps15 Ala448-Thr579 Accession # P42566
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

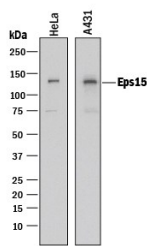
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	0.1 µg/mL	See Below
Immunocytochemistry	1-25 µg/mL	See Below
Intracellular Staining by Flow Cytometry	0.25 µg/10 ⁶ cells	See Below
Simple Western	1 µg/mL	See Below

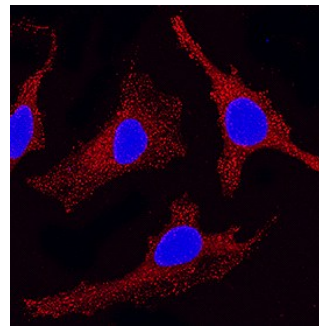
DATA

Western Blot



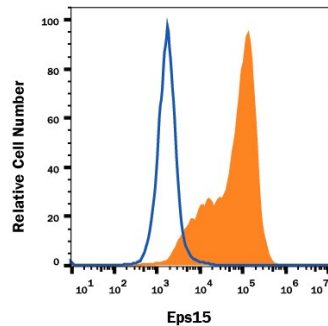
Detection of Human Eps15 by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line and A431 human epithelial carcinoma cell line. PVDF membrane was probed with 0.1 µg/mL of Rabbit Anti-Human Eps15 Monoclonal Antibody (Catalog # MAB8480) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for Eps15 at approximately 140 kDa (as indicated). This experiment was conducted under reducing conditions and using *Immunoblot Buffer Group 1*.

Immunocytochemistry



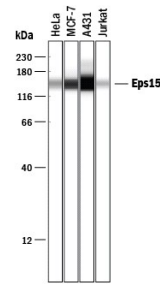
Eps15 in HeLa Human Cell Line. Eps15 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Rabbit Anti-Human Eps15 Monoclonal Antibody (Catalog # MAB8480) at 1 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for *Fluorescent ICC Staining of Cells on Coverslips*.

Intracellular Staining by Flow Cytometry



Detection of Eps15 in U-118-MG Human Cell Line by Flow Cytometry. U-118-MG human glioblastoma/astrocytoma cell line was stained with Rabbit Anti-Human Eps15 Monoclonal Antibody (Catalog # MAB8480, filled histogram) or isotype control antibody (Catalog # AB-105-C, open histogram), followed by Allophycocyanin-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0111). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (Catalog # FC012). View our protocol for *Staining Intracellular Molecules*.

Simple Western



Detection of Human Eps15 by Simple Western™. Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma cell line, MCF-7 human breast cancer cell line, A431 human epithelial carcinoma cell line, and Jurkat human acute T cell leukemia cell line, loaded at 0.2 mg/mL. A specific band was detected for Eps15 at approximately 150 kDa (as indicated) using 1 µg/mL of Rabbit Anti-Human Eps15 Monoclonal Antibody (Catalog # MAB8480). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Eps15 (Epidermal growth factor receptor substrate 15) is a 138-140 kDa member of the Eps family of proteins. Eps15 has a tripartite structure comprising an amino terminal portion, which contains three evolutionary conserved EH protein-protein interaction domains, a central putative coiled-coil region required for constitutive oligomerization, and a carboxy terminal domain containing multiple copies of the amino acid triplet aspartate-prolinephenylalanine that constitute the AP2 binding domain. The carboxy terminal domain also contains two ubiquitin interaction motifs (UIMs), the last of which is indispensable for Eps15 binding to ubiquitin. Eps15 binds to AP-2 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. The EPS15 gene yields two isoforms that are believed to reside in distinct subcellular locations and thus implicated in different facets of endosomal trafficking. Human EPS15 has been mapped to chromosome 1p31- p32, a region displaying several non-random chromosomal abnormalities, including deletions in neuroblastoma and translocation in acute lymphoblastic and myeloid leukemias. Over aa 492-579, human EPS15 shares 76% aa identity with mouse EPS15.