

DESCRIPTION

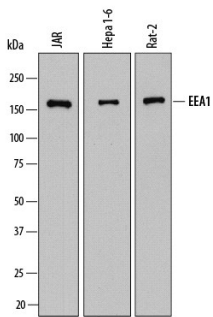
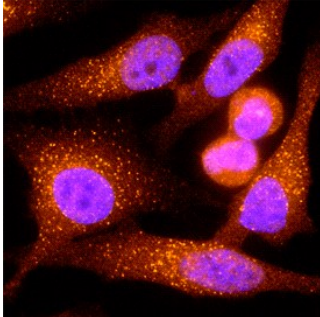
Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat EEA1 in Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human EEA1 Asn1249-Gln1356 Accession # Q15075
Formulation	Lyophilized from a 0.2 µm filtered 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	1 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below

DATA

<p>Western Blot</p> 	<p>Detection of Human, Mouse, and Rat EEA1 by Western Blot. Western blot shows lysates of JAR human choriocarcinoma cell line, Hepa 1-6 mouse hepatoma cell line, and Sheep Anti-Rat-2 rat embryonic fibroblast cell line. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human/Mouse/Rat EEA1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8047) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for EEA1 at approximately 170 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunocytochemistry</p>  <p>EEA1 in HeLa Human Cell Line. EEA1 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Sheep Anti-Human/Mouse/Rat EEA1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF8047) at 1.7 and 5 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to early endosomes. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.</p>
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PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
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

EEA1 (Early Endosome Autoantigen 1; also Endosome-associated protein p162 and Zn-finger FYVE domain-containing protein 2) is a 170-180 kDa protein that serves as an identifying marker for early endosomes. It is ubiquitously expressed, and found in both the cytosol and on cellular membranes. Its activity has been described as that of a tethering factor which links endosomes to endocytic vesicles, allowing for their fusion via a SNARE complex. Normally, EEA1 exists as a homodimer in the cytoplasm and appears to make transient contacts with endosome membrane phosphatidylinositol. When endosome fusion is not required, EEA1 serves as a substrate for p97, promoting EEA1 dissociation and endosome independence. When endosome fusion is required, EEA1 interacts with NSF, resulting in its removal from a large endosome-associated complex and subsequent endosomal vesicle fusion. Human EEA1 is synthesized as a 1411 amino acid (aa) protein that contains one C2H2-type Zn finger region (aa 41-64) and one FYVE Zn finger domain (aa 1352-1410). There is one isoform variant that contains a nine aa substitution for aa 925-1411. Over aa 1249-1356, human EEA1 shares 99% aa sequence identity with mouse EEA1.