Human EphA2 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF3035

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human EphA2 in direct ELISAs and Western blots. In direct ELISAs, less than 45% cross-reactivity with recombinant mouse EphA2 is observed and less than 1% cross-reactivity with recombinant human (rh) EphA1, rhEphA3, rhEphA4, rhEphA5, rhEphA6, rhEphA7 and rhEphA10 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human EphA2 Gln25-Asn534 Accession # P29317
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 either lyophilized or as a 0.2 μm filtered solution in PBS.

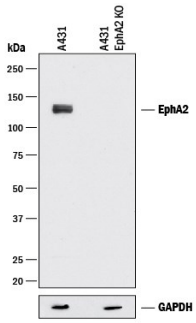
APPLICATIONS

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
Knockout Validated	1 µg/mL	A431 human epithelial carcinoma parental cell line and EphA2 knock out A431 cell line
Western Blot	1 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunohistochemistry	5-15 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

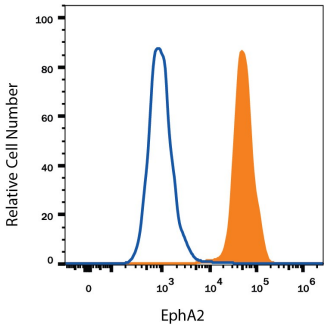
DATA

Western Blot



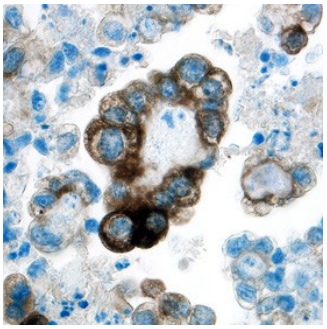
Detection of Human EphA2 by Western Blot. Western blot shows lysates of A431 human epithelial carcinoma parental cell line and EphA2 knock out (KO) A431 cell line. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human EphA2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3035) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for EphA2 at approximately 110 kDa (as indicated), but not detectable in the knockout A431 cell line. GAPDH (Catalog # MAB5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



Detection of EphA2 in A431 Human Cell Line by Flow Cytometry. A431 human epithelial carcinoma cell line was stained with Goat Anti-Human EphA2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3035, filled histogram) or isotype control antibody (Catalog # AB-108-C, open histogram), followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0107). View our protocol for Staining Membrane-associated Proteins.

Immunohistochemistry



EphA2 in Human Ovarian Cancer Tissue. EphA2 was detected in immersion fixed paraffin-embedded sections of human ovarian cancer tissue using Goat Anti-Human EphA2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3035) at 5 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). Specific labeling was localized to the plasma membrane of cancer cells. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

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Human EphA2 Antibody

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Catalog Number: AF3035

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 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.** • 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

EphA2, also known as Eck, Myk2, and Sek2, is a member of the Eph receptor tyrosine kinase family which binds Ephrins A1, 2, 3, 4, and 5 (1-4). A and B class Eph proteins have a common structural organization. The human EphA2 cDNA encodes a 976 amino acid (aa) precursor including a 24 aa signal sequence, a 510 aa extracellular domain (ECD), a 24 aa transmembrane segment, and a 418 aa cytoplasmic domain. The ECD contains an N-terminal globular domain, a cysteine-rich domain, and two fibronectin type III domains (5). The cytoplasmic domain contains a juxtamembrane motif with two tyrosine residues, which are the major autophosphorylation sites, a kinase domain, and a sterile alpha motif (SAM) (5). The ECD of human EphA2 shares 90-94% aa sequence identity with mouse, bovine, and canine EphA2, and approximately 45% aa sequence identity with human EphA1, 3, 4, 5, 7, and 8. EphA2 becomes autophosphorylated following ligand binding (6, 7) and then interacts with SH2 domain-containing PI3-kinase to activate MAPK pathways (8, 9). Reverse signaling is also propagated through the Ephrin ligand. Transcription of EphA2 is dependent on the expression of E-Cadherin (10), and can be induced by p53 family transcription factors (11). EphA2 is upregulated in breast, prostate, and colon cancer vascular endothelium. Its ligand, EphrinA1, is expressed by the local tumor cells (12, 13). In some cases, EphA2 and EphrinA1 are expressed on the same blood vessels (14). EphA2 signaling cooperates with VEGF receptor signaling in promoting endothelial cell migration (13). The gene encoding human EphA2 maps to a region on chromosome 1 which is frequently deleted in neuroectodermal tumors (15).

References:

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