Human EpCAM/TROP-1 Antibody

Antigen Affinity-purified Polyclonal Goat IgG

Catalog Number: AF960

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human EpCAM/TROP-1 in direct ELISAs and Western blots. In direct ELISAs, less than 1% cross-reactivity with recombinant human TROP-2 and approximately 26% cross-reactivity with recombinant mouse EpCAM/TROP-1 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human EpCAM/TROP-1, extracellular domain Gln24-Lys265 Accession # CAA32870
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.

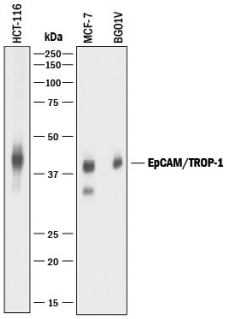
APPLICATIONS

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
Western Blot	0.2 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	0.3-15 µg/mL	See Below
Simple Western	10 µg/mL	A549 human lung carcinoma cell line exosomes, HT-29 human colon adenocarcinoma cell line exosomes, and COLO 205 human colorectal adenocarcinoma cell line whole cell lysate (WCL)
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

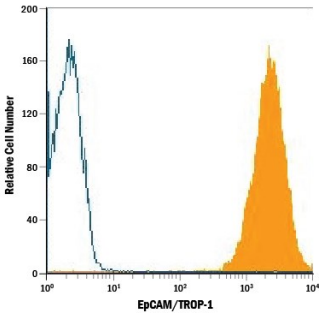
DATA

Western Blot



Detection of Human EpCAM/TROP-1 by Western Blot. Western blot shows lysates of HCT-116 human colorectal carcinoma cell line, MCF-7 human breast cancer cell line, and BG01V human embryonic stem cells. PVDF membrane was probed with 0.2 µg/mL of Goat Anti-Human EpCAM/TROP-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF960) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for EpCAM/TROP-1 at approximately 40 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



Detection of EpCAM/TROP-1 in HT-29 Human Cell Line by Flow Cytometry. HT-29 human colon adenocarcinoma cell line was stained with Goat Anti-Human EpCAM/TROP-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF960, filled histogram) or isotype control antibody (Catalog # AB-108-C, open histogram), followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # F0107).

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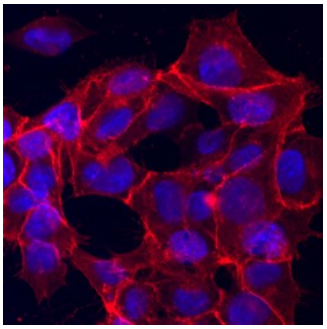
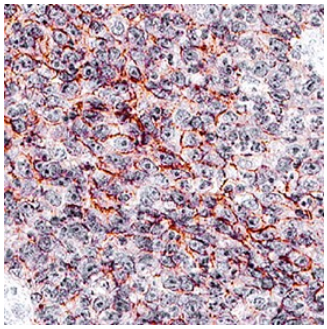
Immunocytochemistry



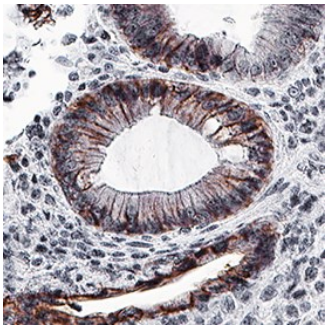
EpCAM/TROP-1 in BG01V Human Embryonic Stem Cells. EpCAM/TROP-1 was detected in immersion fixed BG01V human embryonic stem cells using Goat Anti-Human EpCAM/TROP-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF960) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm and cell membrane. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



EpCAM/TROP-1 in Human Colon. EpCAM/TROP-1 was detected in immersion fixed paraffin-embedded sections of human colon using Goat Anti-Human EpCAM/TROP-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF960) at 0.3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm and plasma membrane. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Immunohistochemistry



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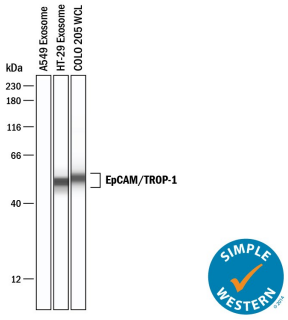
Simple Western



Detection of Human EpCAM/TROP-1 by Simple Western™. Simple Western lane view shows lysates of A549 human lung carcinoma cell line exosomes, HT-29 human colon adenocarcinoma cell line exosomes, and COLO 205 human colorectal adenocarcinoma cell line whole cell lysate (WCL), loaded at 0.2 mg/mL. A specific band was detected for EpCAM/TROP-1 at approximately 52 kDa (as indicated) using 10 µg/mL of Goat Anti-Human EpCAM/TROP-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF960) . This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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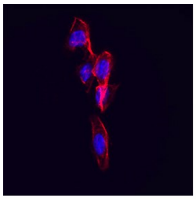
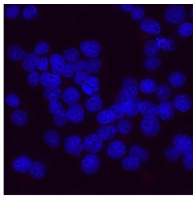
Immunocytochemistry



HT-29 (Positive) cells



Jurkat (Negative) cells

Detection of EpCAM/TROP-1 in HT-29 Human Colon Adenocarcinoma Cell Line (Positive) and Jurkat Human Acute T Cell Leukemia Cell Line (Negative) Cells. EpCAM/TROP-1 was detected in immersion fixed HT-29 human colon adenocarcinoma cell line (positive) and Jurkat human acute T cell leukemia cell line (negative) cells using Goat Anti-Human EpCAM/TROP-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF960) at 5 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cell surface . View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

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

Epithelial Cellular Adhesion Molecule (EpCAM), also known as KS1/4, gp40, GA733-2, 17-1A, and TROP-1, is a 40 kDa transmembrane glycoprotein composed of a 242 amino acid (aa) extracellular domain with two epidermal-growth-factor-like (EGF-like) repeats within the cysteine-rich N-terminal region, a 23 aa transmembrane domain, and a 26 aa cytoplasmic domain. Human and mouse EpCAM share 82% aa sequence identity. In human, EpCAM also shares 49% aa sequence homology with TROP-2/EGP-1. During embryonic development, EpCAM is detected in fetal lung, kidney, liver, pancreas, skin, and germ cells. In adults, human EpCAM is detected in basolateral cell membranes of all simple, pseudo-stratified, and transitional epithelia, but is not detected in normal squamous stratified epithelia, mesenchymal tissue, muscular tissue, neuro-endocrine tissue, or lymphoid tissue (1). EpCAM expression has been found to increase in actively proliferating epithelia tissues and during adult liver regeneration (1, 2). EpCAM expression is also found to increase in human malignant neoplasias, with most carcinoma expressing EpCAM including those of arising from squamous epithelia (1). EpCAM has been shown function as a homophilic Ca^{2+} independent adhesion molecule (3). Homophilic adhesion via EpCAM requires the interaction of both EGF-like repeats, with the first EGF-like repeat mediating reciprocal interaction between EpCAM molecules on opposing cells, while the second repeat is involved in lateral interaction of EpCAM. Lateral interaction of EpCAM lead to the formation of dimers and tetramers (4). During homophilic adhesion the cytoplasmic tail of EpCAM interacts with the actin cytoskeleton via a direct association α-actinin (5).

References:

1. Balzar, M. *et al.* (1999) J. Mol. Med. **77**:699.
2. Boer, C.J. *et al.* (1999) J. Pathol. **188**:201.
3. Litvinow, S.V. *et al.* (1994) J. Cell Biol. **125**:437.
4. Balzar, M. *et al.* (2001) Mol. Cell. Biol. **21**:2570.
5. Balzar, M. *et al.* (1998) Mol. Cell. Biol. **18**:4388.

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