

# Human EpCAM/TROP-1 Antibody

Monoclonal Mouse IgG<sub>2A</sub> Clone # 158210

Catalog Number: MAB960

DESCRIPTION		
Species Reactivity	Human	
Specificity	Detects human EpCAM/TROP-1 in direct ELISAs and Western blots. This antibody detects an epitope found in the extracellular domain between amino acids 136 and 265. In direct ELISAs and Western blots, no cross-reactivity with recombinant human (rh) ALCAM, rhBCAM, rhMCAM, rhNCAM-L1, or recombinant mouse (rm) OCAM is observed. In direct ELISAs, less than 5% cross-reactivity with rmEpCAM/TROP-1 is observed.	
Source	Monoclonal Mouse IgG <sub>2A</sub> Clone # 158210	
Purification	Protein A or G purified from hybridoma culture supernatant	
Immunogen	Mouse myeloma cell line NS0-derived recombinant human EpCAM/TROP-1 Gln24-Lys265 Accession # P16422	
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.				
Western Blot	1 µg/mL	Recombinant Human EpCAM/TROP-1 Fc Chimera (Catalog # 960-EP)		
Immunocytochemistry	8-25 μg/mL	See Below		
Multiplex Immunofluorescence	3-25 μg/mL	Immersion fixed paraffin-embedded sections of human colon		
Immunohistochemistry	8-25 μg/mL	See Below		

#### DATA

### Multiplex Immunofluorescence



Detection of EpCAM/TROP-1 in Human Colon via Multiplex Immunofluorescence staining on COMET™ EpCAM/TROP-1 was detected in immersion fixed paraffin-embedded sections of human colon using Mouse Anti-Human EpCAM/TROP-1 Monoclonal Antibody (Catalog # MAB960) at 1µg/mL at 37 ° Celsius for 4 minutes. Before incubation with the primary antibody, tissue underwent an allin-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIFR Buffer H (pH 9). Tissue was stained using the Alexa Fluor™ 647 Goat anti-Mouse IgG Secondary Antibody at 1:200 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR647MS) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the membrane. Protocol available in COMET™ Panel Builder.

### Immunocytochemistry



#### Human Cell Line. EpCAM/TROP-1 was detected in immersion fixed HT-29 human colon adenocarcinoma cell line using Mouse Anti-Human EpCAM/TROP-1 Monoclonal Antibody (Catalog # MAB960) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 493conjugated Anti-Mouse IgG Secondary Antibody (green; Catalog # NL009) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

EpCAM/TROP-1 in HT-29

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Global | bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL: 1.612.379.2956 USA | TEL: 800.343.7475 Canada | TEL: 855.668.8722 Europe | Middle East | Africa TEL: +44.0.1235.529449 China | info.cn@bio-techne.com TEL: 400.821.3475

# biotechne **R**DSYSTEMS

# Human **EpCAM/TROP-1** Antibody

Monoclonal Mouse IgG2A Clone # 158210 Catalog Number: MAB960



#### EpCAM/TROP-1 in HT-29 Human Cell Line.

EpCAM/TROP-1 was detected in immersion fixed HT-29 human colon adenocarcinoma cell line using Mouse Anti-Human EpCAM/TROP-1 Monoclonal Antibody (Catalog # MAB960) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557conjugated Anti-Mouse IgG Secondary Antibody (yellow; Catalog # NL007) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

#### Immunohistochemistry



#### EpCAM/TROP-1 in Human Adenocarcinoma. EpCAM/TROP-1 was detected in immersion fixed paraffinembedded sections of human adenocarcinoma using Mouse Anti-Human EpCAM/TROP-1 Monoclonal Antibody (Catalog # MAB960) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS002) and counterstained with hematoxylin (blue). Specific staining was localized to cancer cells. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

#### Immunocytochemistry



HT-29 (Positive) cells

Daudi (Negative) cells

Detection of EpCAM/TROP-1 in HT-29 Human Colon Adenocarcinoma Cell Line (Positive) and Daudi Human Burkitt's Lymphoma Cell Line (Negative) Cells . EpCAM/TROP-1 was detected in immersion fixed HT-29 human colon adenocarcinoma cell line (positive) and Daudi human Burkitt's lymphoma cell line (negative) cells using Mouse Anti-Human EpCAM/TROP-1 Monoclonal Antibody (Catalog # MAB960) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cell surface and cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

FREFARATION AND STORAGE		
Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.	
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.	
Stability & Storage	<ul> <li>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</li> <li>12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>	

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### 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 squamousal epithelia (1). EpCAM has been shown function as a homophilic Ca<sup>2+</sup> 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  $\alpha$ -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.