

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human ULBP-4/RAET1E in direct ELISAs and Western blots. In Western blots, approximately 20% cross-reactivity with recombinant human (rh) ULBP-2 is observed and no cross-reactivity with rhULBP-1 or rhULBP-3 is observed.
Source	Monoclonal Mouse IgG _{2B} Clone # 709116
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Mouse myeloma cell line NS0-derived recombinant human ULBP-4/RAET1E Gly30-Asp225 Accession # Q8TD07
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose.

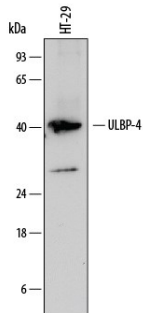
APPLICATIONS

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
Flow Cytometry	0.25 µg/10 ⁶ cells	See Below
Immunohistochemistry	8-25 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.	

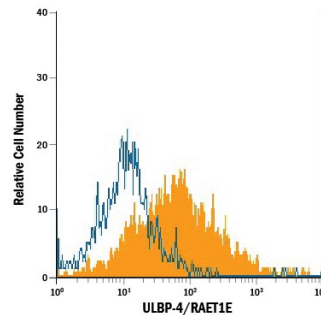
DATA

Western Blot



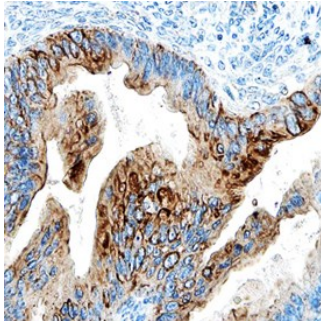
Detection of Human ULBP-4/RAET1E by Western Blot. Western blot shows lysates of HT-29 human colon adenocarcinoma cell line. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human ULBP-4/ RAET1E Monoclonal Antibody (Catalog # MAB6285) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for ULBP-4/RAET1E at approximately 40 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



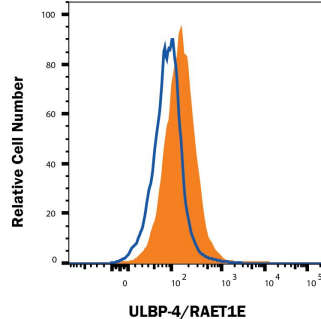
Detection of ULBP-4/RAET1E in HepG2 Human Cell Line by Flow Cytometry. HepG2 human hepatocellular carcinoma cell line was stained with Mouse Anti-Human ULBP-4/RAET1E Monoclonal Antibody (Catalog # MAB6285, filled histogram) or isotype control antibody (Catalog # MAB0041, open histogram), followed by Allophycocyanin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0101B).

Immunohistochemistry



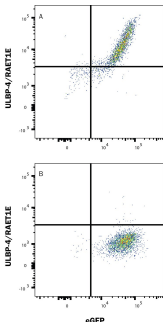
ULBP-4/RAET1E in Human Ovarian Cancer Tissue. ULBP-4/RAET1E was detected in immersion fixed paraffin-embedded sections of human ovarian cancer tissue using Mouse Anti-Human ULBP-4/ RAET1E Monoclonal Antibody (Catalog # MAB6285) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS002) and counter-stained with hematoxylin (blue). Specific staining was localized to plasma membranes of epithelial cells. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

Flow Cytometry



Detection of ULBP-4/RAET1E in U87-MG cells by Flow Cytometry. U87-MG cells were stained with Mouse Anti-Human ULBP-4/RAET1E Monoclonal Antibody (Catalog # MAB6285, filled histogram) or isotype control antibody (Catalog # MAB0041, open histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0102B). View our protocol for [Staining Membrane-associated Proteins](#).

Flow Cytometry



Detection of ULBP-4/RAET1E in HEK293 cells transfected with Human ULBP-4 vs irrelevant (B) by Flow Cytometry HEK293 cells transfected with Human ULBP-4 (A) vs irrelevant (B) were stained with Mouse Anti-Human ULBP-4/RAET1E Monoclonal Antibody (Catalog # MAB6285) followed by Allophycocyanin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0101B). View our protocol for [Staining Membrane-associated Proteins](#).

PREPARATION AND STORAGE

Reconstitution Sterile PBS to a final concentration of 0.5 mg/mL.

Shipping The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.

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

ULBP-4 (cytomegalovirus glycoprotein UL16 binding protein 4), also called RAET1E (retinoic acid early transcript 1E), Leta1 (lymphocyte effector cell toxicity activating ligand) and NKG2DL4 (NKG2D ligand 4), is a 40-50 kDa member of the ULBP/RAET1 family of cell surface proteins that function as ligands for NKG2D (1-6). While most family members are GPI-anchored, only ULBP-4/RAET1E and ULBP-5/RAET1G express a transmembrane form (1, 4, 6, 7). Human ULBP-4 mRNA encodes 263 amino acids (aa) including a 30 aa signal sequence, a 195 aa extracellular domain (ECD), a 23 aa transmembrane domain, and a 15 aa cytoplasmic sequence. A soluble 35 kDa form diverges at aa 208 and is thought to antagonize the transmembrane form (5). Other potential splice variants of 220, 227 and 280 aa are transmembrane proteins (8). Within the ECD, ULBP-4 shares 34-41% aa sequence identity with family members (1, 7). Rodent NKG2D ligands Rae-1 α - ϵ are, like human ULBP and MIB proteins, distantly related to MHC class I proteins, but none of the families share significant sequence identity (2, 4). Low expression of ULBP-4 mRNA is found in normal tissues, with high expression variably reported in the small intestine (3) and skin (4). Expression is stimulated by TNF- α and down-regulated by retinoic acid (3). ULBP-4 is abnormally expressed on most colon cancer and some other tumor cell lines and virus-infected peripheral blood cells (3, 6).

ULBP-4 binds and costimulates NKG2D-expressing effector cells including NK cells, NKT cells, $\gamma\delta$ T cells, and CD8⁺ $\alpha\beta$ T cells, activating cytolytic activity and/or cytokine production (3, 4, 7). In some $\gamma\delta$ T cells, direct ULBP-4 binding to both TCR $\gamma\delta$ and NKG2D has been demonstrated (6). ULBP-4 is also thought to function as a minor histocompatibility antigen in humans (1).

References:

1. Radosavljevic, M. *et al.* (2002) *Genomics* **79**:114.
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4. Chalupny, N.J. *et al.* (2003) *Biochem. Biophys. Res. Commun.* **305**:129.
5. Cao, W. *et al.* (2007) *J. Biol. Chem.* **282**:18922.
6. Kong, Y. *et al.* (2009) *Blood* **114**:310.
7. Bacon, L. *et al.* (2004) *J. Immunol.* **173**:1078.
8. Cao, W. *et al.* (2008) *Int. Immunol.* **20**:981.