Human B7-H3 Antibody

Recombinant Monoclonal Rabbit IgG Clone # 3108D
Catalog Number: MAB11721

DESCRIPTION

Species Reactivity	Human
Specificity	Detects recombinant human B7-H3 protein in Direct ELISA.
Source	Recombinant Monoclonal Rabbit IgG Clone # 3108D
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Mouse myeloma cell line, NS0-derived human B7-H3 Leu29-Pro245 Accession # Q5ZPR3
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose.

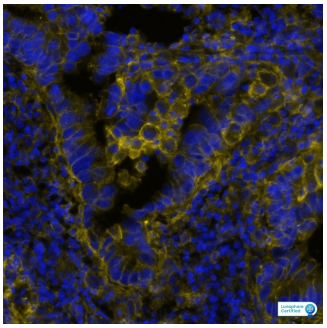
APPLICATIONS

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	0.25 µg/mL	JAR human choriocarcinoma cell line and LNCaP human prostate cancer cell line
Multiplex Immunofluorescence	1 µg/mL	Immersion fixed paraffin-embedded sections of human lung tumor
Immunohistochemistry	0.5-10 µg/mL	Immersion fixed paraffin-embedded sections of human liver cancer and lung cancer
Simple Western	2-10 µg/mL	LNCaP human prostate cancer cell line

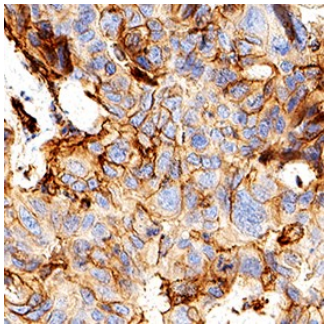
DATA

Multiplex Immunofluorescence



B7-H3 in Human Lung Tumor via seqIF™ staining on COMET™ B7-H3 was detected in immersion fixed paraffin-embedded sections of human Lung Tumor using Rabbit Anti-Human B7-H3, Monoclonal Antibody (Catalog #MAB11721) at 1ug/mL at 37 ° Celsius for 4 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9; Epredia Catalog # TA-999-DHBH). Tissue was stained using the Alexa Fluor™ Plus 647 Goat anti-Rabbit IgG Secondary Antibody at 1:200 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR647RB) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the membrane. Protocol available in COMET™ Panel Builder.

Immunohistochemistry



Detection of B7-H3 in Human Liver Cancer. B7-H3 was detected in immersion fixed paraffin-embedded sections of human liver cancer using Rabbit Anti-Human B7-H3 Monoclonal Antibody (Catalog # MAB11721) at 0.5 µg/ml for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003) or the HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using VisUCyte Antigen Retrieval Reagent-Basic (Catalog # VCTS021). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to the membrane. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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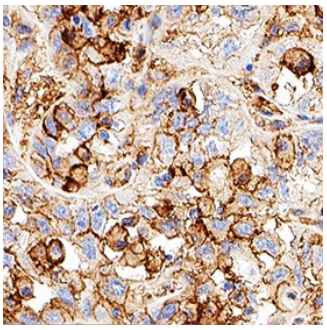
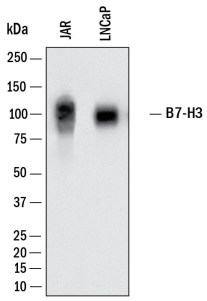


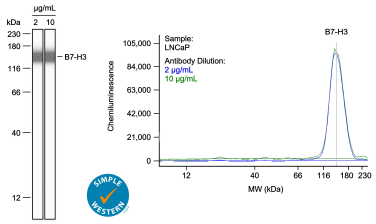
Detection of B7-H3 in Human Lung Cancer. B7-H3 was detected in immersion fixed paraffin-embedded sections of human lung cancer using Rabbit Anti-Human B7-H3 Monoclonal Antibody (Catalog # MAB11721) at 0.5 µg/ml for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003) or the HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using VisUCyte Antigen Retrieval Reagent-Basic (Catalog # VCTS021). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to the membrane. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Western Blot



Detection of Human B7-H3 by Western Blot. Western Blot shows lysates of JAR human choriocarcinoma cell line and LNCaP human prostate cancer cell line. PVDF membrane was probed with 0.25 µg/ml of Rabbit Anti-Human B7-H3 Monoclonal Antibody (Catalog # MAB11721) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for B7-H3 at approximately 90-110 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

Simple Western



Detection of Human B7-H3 by Simple Western™. Left: Simple Western lane view shows lysates of LNCaP human prostate cancer cell line, loaded at 0.1 mg/ml. A specific band was detected for B7-H3 at approximately 149 kDa (as indicated) using both 2 µg/ml and 10 µg/ml of Rabbit Anti-Human B7-H3 Monoclonal Antibody (Catalog # MAB11721) followed by HRP-conjugated Goat Anti-Rabbit Secondary Antibody (Catalog # 042-206). This experiment was conducted under reducing conditions and using the 12-230kDa separation system. **Right:** Simple Western electropherogram showing the same Rabbit Anti-Human B7-H3 Monoclonal Antibody (Catalog # MAB11721) tested at 2 µg/ml (blue line) and 10 µg/ml (green line) in the LNCaP human prostate cancer cell line.

PREPARATION AND STORAGE

Reconstitution	Reconstitute lyophilized material at 0.2 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	**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.

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BACKGROUND

Human B7 homolog 3 (B7-H3) is a member of the B7 family of immune proteins that provide signals for regulating immune responses (1-3). Other family members include B7-1, B7-2, B7-H2, PD-L1 (B7-H1), and PD-L2. B7 proteins are immunoglobulin (Ig) superfamily members with extracellular Ig-V-like and Ig-C-like domains and short cytoplasmic domains. Among the family members, they share about 20-40% amino acid (aa) sequence identity. The cloned human B7-H3 cDNA encodes a 316 aa type I membrane precursor protein with a putative 28 aa signal peptide, a 217 aa extracellular region containing one V-like and one C-like Ig domain, a transmembrane region, and a 45 aa cytoplasmic domain. An isoform of human B7-H3 containing a four-Ig-like domain extracellular region has also been identified. Human B7-H3 is not expressed on resting B cells, T cells, monocytes or dendritic cells, but is induced on dendritic cells and monocytes by inflammatory cytokines. B7-H3 expression is also detected on various normal tissues and in some tumor cell lines. Human B7-H3 does not bind any known members of the CD28 family of immunoreceptors. However, B7-H3 has been shown to bind an unidentified counter-receptor on activated T cells to costimulate the proliferation of CD4+ or CD8+ T cells. B7-H3 has also been found to enhance the induction of primary cytotoxic T lymphocytes and stimulate IFN-γ production (1-3).

References:

1. Chapoval, A.I. *et al*. (2001) Nat. Immunol. **2**:269.
2. Sharpe, A.H. and G.J. Freeman (2002) Nat. Rev. Immunol. **2**:116.
3. Coyle, A. and J. Gutierrez-Ramos (2001) Nat. Immunol. **2**:203.

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