Human B7-H3 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1027

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human B7-H3 in direct ELISAs and Western blots. In direct ELISAs, approximately 5% cross-reactivity with recombinant human (rh) B7-H2 is observed, and less than 1% cross-reactivity with rhB7-1, rhB7-2, rhB7-H1, and rhPD-L2 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human B7-H3 Leu29-Pro245 Accession # NP_079516
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.

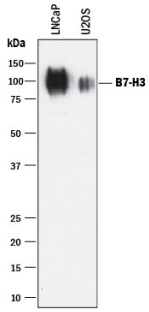
APPLICATIONS

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	1 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	10 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.	
Knockout Validated	B7-H3 is specifically detected in U2OS human osteosarcoma parental cell line but is not detectable in B7-H3 knockout U2OS cell line.	

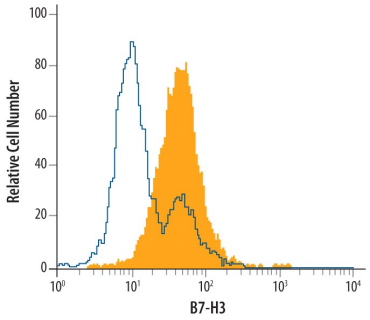
DATA

Western Blot



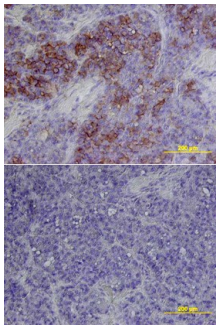
Detection of Human B7-H3 by Western Blot. Western blot shows lysates of LNCaP human prostate cancer cell line and U2OS human osteosarcoma cell line. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human B7-H3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1027) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for B7-H3 at approximately 90-110 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



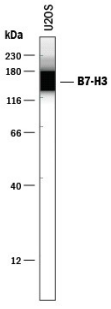
Detection of B7-H3 in PC-3 Human Cell Line by Flow Cytometry. PC-3 human prostate carcinoma cells were stained with Goat Anti-Human B7-H3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1027, filled histogram) or control antibody (Catalog # AB-108-C, open histogram), followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0107).

Immunohistochemistry



B7-H3 in Human Melanoma. B7-H3 was detected in immersion fixed paraffin-embedded sections of human melanoma using Goat Anti-Human B7-H3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1027) at 15 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). Lower panel shows a lack of labeling if primary antibodies are omitted and tissue is stained only with secondary antibody followed by incubation with detection reagents. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Simple Western



Detection of Human B7-H3 by Simple Western™. Simple Western lane view shows lysates of U2OS human osteosarcoma cell line, loaded at 0.2 mg/mL. A specific band was detected for B7-H3 at approximately 120-160 kDa (as indicated) using 10 µg/mL of Goat Anti-Human B7-H3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1027) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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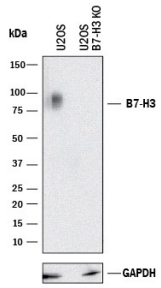
Knockout Validated



Western Blot Shows Human B7-H3 Specificity by Using Knockout Cell Line. Western blot shows lysates of U2OS human osteosarcoma parental cell line and B7-H3 knockout U2OS cell line (KO). PVDF membrane was probed with 1 µg/mL of Goat Anti-Human B7-H3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1027) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for B7-H3 at approximately 95 kDa (as indicated) in the parental U2OS cell line, but is not detectable in knockout U2OS cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

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

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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