

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 as a 0.2 µm filtered solution in PBS.

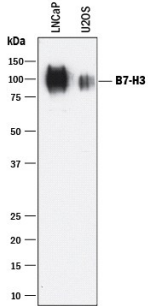
APPLICATIONS

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.	

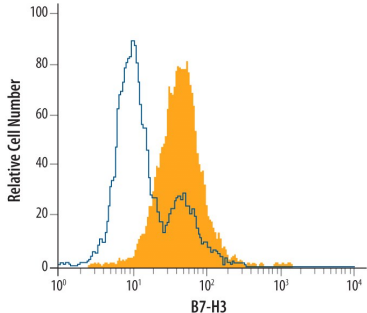
DATA

Western Blot



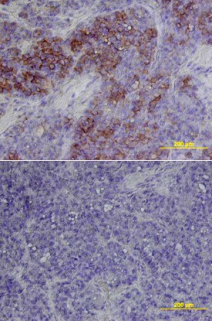
Detection of Human B7-H3 by Western Blot. Western blot shows lysates of LNCaP human prostate cancer cell line and U2OS human osteosarcoma cell line. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human B7-H3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1027) followed by HRP-conjugated 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



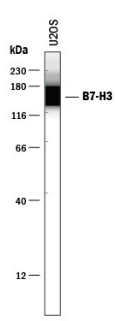
Detection of B7-H3 in PC-3 Human Cell Line by Flow Cytometry. PC-3 human prostate carcinoma cells were stained with Goat Anti-Human B7-H3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1027, filled histogram) or control antibody (Catalog # AB-108-C, open histogram), followed by Phycoerythrin-conjugated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

Immunohistochemistry




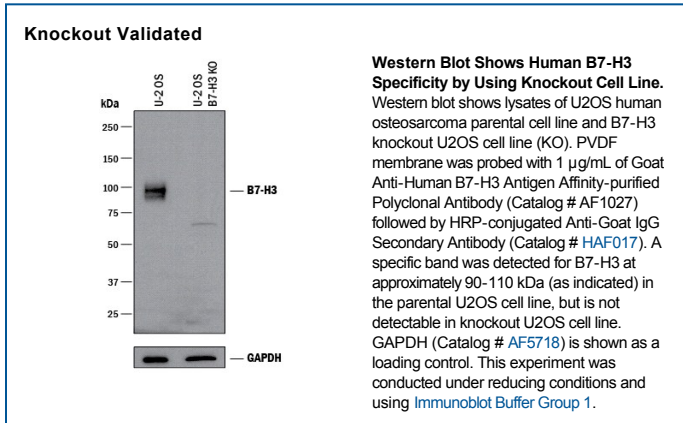
B7-H3 in Human Melanoma. B7-H3 was detected in immersion fixed paraffin-embedded sections of human melanoma using Goat Anti-Human B7-H3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1027) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining 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.





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. <ul style="list-style-type: none"> ● 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.