

**DESCRIPTION**

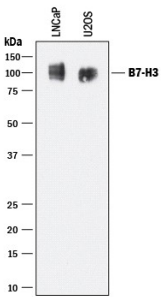
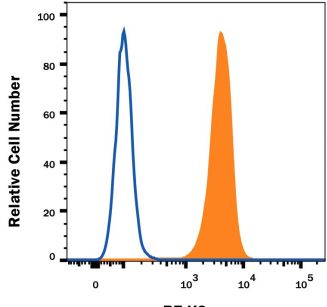
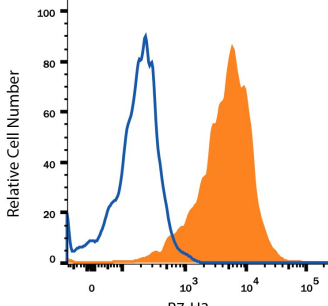
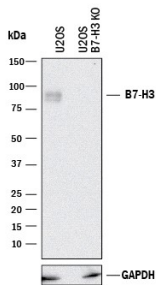
<b>Species Reactivity</b>	Human
<b>Specificity</b>	Detects human B7-H3 in direct ELISAs and Western blots. In Western blots, no cross-reactivity with recombinant human (rh) B7-H1, rhB7-H2, rhB7-1, rhB7-2, or recombinant mouse PD-L2 is observed.
<b>Source</b>	Monoclonal Mouse IgG <sub>1</sub> Clone # 185504
<b>Purification</b>	Protein A or G purified from hybridoma culture supernatant
<b>Immunogen</b>	Mouse myeloma cell line NS0-derived recombinant human B7-H3 Leu29-Pro245 Accession # NP_079516
<b>Formulation</b>	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.

	<b>Recommended Concentration</b>	<b>Sample</b>
<b>Western Blot</b>	2 µg/mL	See Below
<b>Flow Cytometry</b>	0.25 µg/10 <sup>6</sup> cells	See Below
<b>CyTOF-ready</b>	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
<b>Knockout Validated</b>	B7-H3 is specifically detected in U2OS human osteosarcoma parental cell line but is not detectable in B7-H3 knockout U2OS cell line.	

**DATA**

<p><b>Western Blot</b></p>  <p><b>Detection of Human B7-H3 by Western Blot.</b> Western blot shows lysates of LNCaP human prostate cancer cell line and U2OS human osteosarcoma cell line. PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human B7-H3 Monoclonal Antibody (Catalog # MAB1027) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). 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.</p>	<p><b>Flow Cytometry</b></p>  <p><b>Detection of B7-H3 in PC-3 Human Cell Line by Flow Cytometry.</b> PC-3 human prostate cancer cell line was stained with Mouse Anti-Human B7-H3 Monoclonal Antibody (Catalog # MAB1027, filled histogram) or isotype control antibody (Catalog # MAB002, open histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG F(ab')<sub>2</sub> Secondary Antibody (Catalog # F0102B).</p>
<p><b>Flow Cytometry</b></p>  <p><b>Detection of B7-H3 in Human Dendritic Cells by Flow Cytometry.</b> Human monocyte-derived dendritic cells were stained with Mouse Anti-Human B7-H3 Monoclonal Antibody (Catalog # MAB1027, filled histogram) or isotype control antibody (Catalog # MAB002, open histogram), followed by Allophycocyanin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0101B).</p>	<p><b>Knockout Validated</b></p>  <p><b>Western Blot Shows Human B7-H3 Specificity by Using Knockout Cell Line.</b> Western blot shows lysates of U2OS human osteosarcoma parental cell line and B7-H3 knockout U2OS cell line (KO). PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human B7-H3 Monoclonal Antibody (Catalog # MAB1027) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). 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 # MAB5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>

**PREPARATION AND STORAGE**

<b>Reconstitution</b>	Reconstitute at 0.5 mg/mL in sterile PBS.
<b>Shipping</b>	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
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"><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>

**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- $\gamma$  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.