

DESCRIPTION	
Species Reactivity	Mouse
Specificity	Detects mouse B7-H3 in direct ELISAs and Western blots. In direct ELISAs and Western blots, approximately 50% cross-reactivity with recombinant human (rh) B7-H3 is observed, and less than 1% cross-reactivity with recombinant mouse (rm) B7-H1, rmB7-H2 and rmB7-1 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant mouse B7-H3 Val29-Phe244 Accession # Q8VE98
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
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
Neutralization	Measured by its ability to neutralize B7-H3-induced proliferation in mouse CD3 ⁺ T cells. The Neutralization Dose (ND ₅₀) is typically 1.5-7.5 µg/mL in the presence of 2 µg/well Recombinant Mouse B7-H3 and 100 ng/mL Hamster Anti-Mouse CD3ε Monoclonal Antibody (Catalog # MAB484).	

DATA

Western Blot

Detection of Mouse B7-H3 by Western Blot. Western blot shows lysates of MEF mouse embryonic feeder cells, P19 mouse embryonal carcinoma cell line, NIH-3T3 mouse embryonic fibroblast cell line, C2C12 mouse myoblast cell line, and 3T3-L1 mouse embryonic fibroblast adipose-like cell line. PVDF membrane was probed with 1 µg/mL of Goat Anti-Mouse B7-H3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1397) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for B7-H3 at approximately 45-55 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Neutralization

Proliferation Induced by B7-H3 and Neutralization by Mouse B7-H3 Antibody. Recombinant Mouse B7-H3 enhances proliferation in mouse CD3⁺ T cells in the presence of 100 ng/mL Hamster Anti-Mouse CD3ε Monoclonal Antibody (Catalog # MAB484) in a dose-dependent manner (orange line), as measured by the Resazurin (Catalog # AR002). Proliferation elicited by Recombinant Mouse B7-H3 (2 µg/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Mouse B7-H3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1397). The ND₅₀ is typically 1.5-7.5 µg/mL.

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

T cells require a signal induced by the engagement of the T cell receptor and a "co-stimulatory" signal(s) through distinct T cell surface molecules for optimal T cell expansion and activation. Members of the B7 superfamily of counter-receptors were identified by their ability to interact with co-stimulatory molecules found on the surface of T cells. Members of the B7 superfamily include B7-1 (CD80), B7-2 (CD86), B7-H1 (PD-L1), B7-H2 (B7RP-1), B7-H3, and PD-L2 (1). B7-H3 is expressed at very high levels in immature dendritic cells at moderate levels on mature dendritic cells, LPS stimulated immature dendritic cells and LPS stimulated monocytes, and at low levels on resting monocytes. B7-H3 binds to activated T cells via an as-of-yet identified receptor. B7-H3 co-stimulates proliferation of T cells and interferon- γ (IFN- γ) production and enhances the induction of cytotoxic T cells. B7-H3 shares 20-27% amino acid (aa) identity with other B7 family members (2). Murine B7-H3 is a 259 aa protein containing an extracellular domain, a transmembrane domain and a cytoplasmic domain. Mouse and human B7-H3 share 87% aa identity (3).

References:

1. Coyle, A.J. and J.-C. Gutierrez-Ramos (2001) *Nature Immunol.* **2**:203.
2. Chapoval, A.I. *et al.* (2001) *Nature Immunol.* **2**:269.
3. Sun, M. *et al.* (2002) *J. Immunol.* **168**:6294.