

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects mouse B7-H4 in direct ELISAs. Detects human and mouse B7-H4 in Western blots.
Source	Recombinant Monoclonal Rabbit IgG Clone # 2319B
Purification	Protein A or G purified from cell culture supernatant
Immunogen	Mouse myeloma cell line, NS0-derived mouse B7-H4 Phe29-Pro258 Accession # Q7TSP5
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.

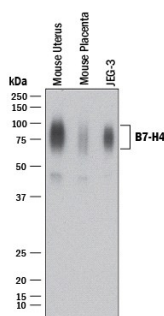
APPLICATIONS

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	5-25 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.	

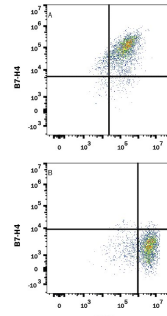
DATA

Western Blot



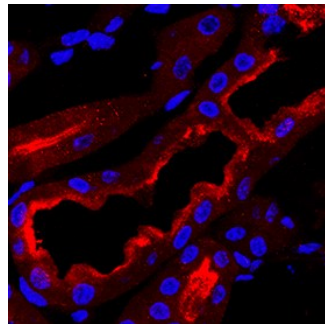
Detection of human and mouse B7-H4 by Western Blot. Western blot shows lysates of mouse uterus tissue, mouse placenta tissue, and JEG-3 human epithelial choriocarcinoma cell line. PVDF membrane was probed with 1 µg/mL of Rabbit Anti-Human/Mouse B7-H4 Monoclonal Antibody (Catalog # MAB21541) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for B7-H4 at approximately 50-80 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



Detection of B7-H4 in HEK293 Human Cell Line Transfected with Mouse B7-H4 and eGFP by Flow Cytometry. HEK293 human embryonic kidney cell line transfected with either (A) mouse B7-H4 or (B) irrelevant protein and eGFP was stained with Rabbit Anti-Human/Mouse B7-H4 Monoclonal Antibody (Catalog # MAB21541) followed by APC-conjugated Goat anti-Rabbit IgG Secondary Antibody (Catalog # F0111). Quadrant markers were set based on Rabbit IgG control antibody staining (Catalog # MAB1050). View our protocol for [Staining Membrane-associated Proteins](#).

Immunohistochemistry



B7-H4 in Mouse Kidney. B7-H4 was detected in perfusion fixed frozen sections of mouse kidney using Rabbit Anti-Human/Mouse B7-H4 Monoclonal Antibody (Catalog # MAB21541) at 10 µg/mL overnight at 4 °C. Tissue was stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to cell surfaces of epithelial cells in convoluted tubules. View our protocol for [Fluorescent IHC Staining of Frozen Tissue Sections](#).

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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

B7-H4, also known as B7x and B7S1, is a 50-80 kDa glycosylated member of the B7 family of immune co-stimulatory proteins (1, 2). Mature mouse B7-H4 consists of a 230 amino acid (aa) extracellular domain (ECD) with one Ig-like V-set domain and one Ig-like C2-set domain which is followed by a hydrophobic C-terminal region (3-5). Within the ECD, mouse B7-H4 shares 90% and 99% aa sequence identity with human and rat B7-H4, respectively. It shares 21%-29% aa sequence identity with mouse B7-1, B7-2, B7-H1, B7-H2, B7-H3, and PD-L2. B7-H4 is expressed on the surface of activated lymphocytes, macrophages, monocytes, dendritic cells, epithelial cells, and bone marrow-derived mesenchymal stem cells (4-8). Its binding to activated T cells dampens T cell responses and induces cell cycle arrest in the T cell (3-5). Reverse signaling can induce either cell cycle arrest or apoptosis in the B7-H4 expressing cell (9, 10). B7-H4 is up-regulated in several carcinomas in correlation with tumor progression and metastasis (2, 7, 11, 12). A soluble form of B7-H4 is elevated in the serum of ovarian cancer, renal cell carcinoma, and rheumatoid arthritis patients, also in correlation with advanced disease status (13-15). Soluble B7-H4 functions as a decoy molecule that blocks the inhibitory influence of B7-H4 on immune activation (15). Despite evidence for the involvement of B7-H4 in immune regulation, mice deficient in its expression do not show significant immune deficiencies, suggesting compensation by other molecules *in vivo* (16).

References:

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