Human B7-H4 Antibody

Recombinant Monoclonal Rabbit IgG Clone # 2318A
Catalog Number: MAB65764

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human B7-H4 in direct ELISAs.
Source	Recombinant Monoclonal Rabbit IgG Clone # 2318A
Purification	Protein A or G purified from cell culture supernatant
Immunogen	Mouse myeloma cell line, NS0-derived human B7-H4 Met1-Ser988 Accession # Q7Z7D3
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.

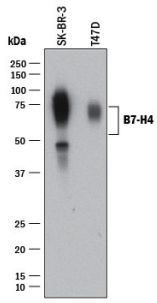
APPLICATIONS

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	3-25 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.	

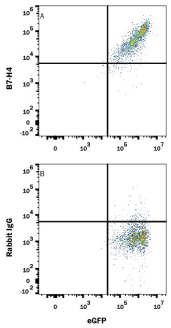
DATA

Western Blot



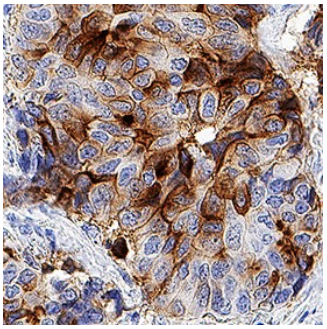
Detection of human B7-H4 by Western Blot. Western blot shows lysates of SK-BR-3 human breast cancer cell line and T47D human breast cancer cell line. PVDF membrane was probed with 1 µg/mL of Rabbit Anti-Human B7-H4 Monoclonal Antibody (Catalog # MAB65764) followed by HRP-conjugated Anti-Rabbit IgG Secondary 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 Human B7-H4 and eGFP by Flow Cytometry. HEK293 human embryonic kidney cell line transfected with human B7-H4 and eGFP was stained with either (A) Rabbit Anti-Human B7-H4 Monoclonal Antibody (Catalog # MAB65764) or (B) Rabbit IgG control antibody (Catalog # MAB1050) followed by APC-conjugated Goat anti-Rabbit IgG Secondary Antibody (Catalog # F0111). View our protocol for Staining Membrane-associated Proteins.

Immunohistochemistry



B7-H4 in Human Breast Cancer Tissue. B7-H4 was detected in immersion fixed paraffin-embedded sections of human breast cancer tissue using Rabbit Anti-Human B7-H4 Monoclonal Antibody (Catalog # MAB65764) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cell surfaces in cancer cells. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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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.** • 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 VTCN1, B7x and B7S1, is a 50-80 kDa glycosylated member of the BTN/MOG family of immunomodulatory protein (1, 2). Mature human B7-H4 consists of a 235 amino acid (aa) extracellular domain (ECD) with one Ig-like V-set domain and one Ig-like C2-set domain, a 21 aa transmembrane segment, and a 2 aa cytoplasmic tail (3-5). Within the ECD, human B7-H4 shares 90% aa sequence identity with mouse and rat B7-H4. It shares 22%-28% aa sequence identity with human B7-1, B7-2, B7-H1, B7-H2, B7-H3, and PD-L2. Alternate splicing of human B7-H4 generates an additional isoform that lacks the first Ig-like domain. 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). Following binding to activated T cells, B7-H4 serves as a co-inhibitor of the T cell response. This is accomplished by reverse signaling that can induce either cell cycle arrest, or apoptosis in B7-H4 expressing cells (3-5, 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).

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