

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects mouse VISTA/B7-H5/PD-1H in direct ELISAs and Western blots. In direct ELISAs, approximately 25% cross-reactivity with recombinant human VISTA/B7-H5/PD-1H is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant mouse VISTA/B7-H5/PD-1H Phe33-Ala191 Accession # Q9D659
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.

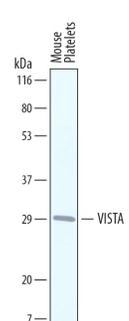
APPLICATIONS

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
Immunocytochemistry	5-15 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.	

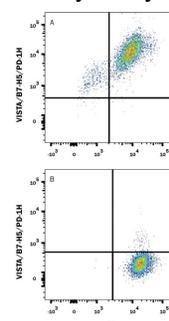
DATA

Western Blot



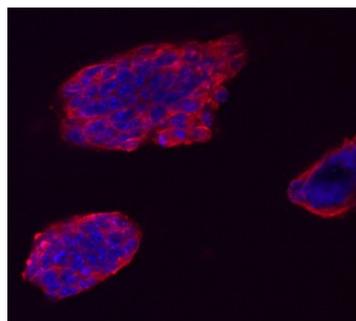
Detection of VISTA/B7-H5/PD-1H by Western Blot. Western blot shows lysates of mouse platelets. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Mouse VISTA/B7-H5/PD-1H Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7005) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for VISTA/B7-H5/PD-1H at approximately 30 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 8.

Flow Cytometry



Detection of VISTA/B7-H5/PD-1H in HEK293 Human Cell Line Transfected with Mouse VISTA and eGFP by Flow Cytometry. HEK293 human embryonic kidney cell line transfected with either (A) mouse VISTA or (B) irrelevant transfectants and eGFP was stained with Sheep Anti-Mouse VISTA/B7-H5/PD-1H Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7005) followed by Allophycocyanin-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # F0127). Quadrant markers were set based on control antibody staining (Catalog # 5-001-A). View our protocol for [Staining Membrane-associated Proteins](#).

Immunocytochemistry



VISTA/B7-H5/PD-1H in D3 Mouse Cell Line. VISTA/B7-H5/PD-1H was detected in immersion fixed D3 mouse embryonic stem cell line using Sheep Anti-Mouse VISTA/B7-H5/PD-1H Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7005) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to cell surfaces and cytoplasm. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
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

Platelet Receptor Gi24 (also known as VISTA, B7-H5, SISP1, C10orf54 and , Dies1 [Differentiation of ESC-1]) is a 55-65 kDa member of the Ig superfamily. It is a transmembrane molecule expressed in bone, on embryonic stem cells (ESCs), and on tumor cell surfaces. On ESCs, Gi24 appears to positively interact with BMP4, potentiating BMP signaling and the transition from an undifferentiated to a differentiated state. On tumor cells, Gi24 both promotes MT1-MMP expression and activity, and serves as a substrate for MT1-MMP. This increases the potential for cell motility. Mature mouse Gi24 is a 277 amino acid (aa) type I transmembrane glycoprotein (aa 33-309). It contains a 149 aa extracellular region (aa 33-191) with one V-type Ig-like domain (aa 33-161) and a 97 aa cytoplasmic domain. Based on human Gi24, mouse Gi24 will likely undergo proteolytic cleavage by MT1-MMP, generating a soluble 30 kDa extracellular fragment, plus a 25-30 kDa membrane-bound fragment. There are two potential isoform variants. One contains a deletion of aa 127-187, while another shows an alternative start site at Met82. Over aa 33-191, mouse Gi24/Dies1 shares 78% and 70% aa identity with rat and human Gi24, respectively.