Human VISTA/B7-H5/PD-1H Antibody

Recombinant Monoclonal Rabbit IgG Clone # 3180A
Catalog Number: MAB11712

DESCRIPTION

Species Reactivity	Human
Specificity	Detects a synthetic peptide specific for human VISTA/B7-H5/PD-1H around amino acid 100 in Direct ELISA.
Source	Recombinant Monoclonal Rabbit IgG Clone # 3180A
Purification	Protein A or G purified from cell culture supernatant
Immunogen	Synthetic peptide Accession # Q9H7M9
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose.

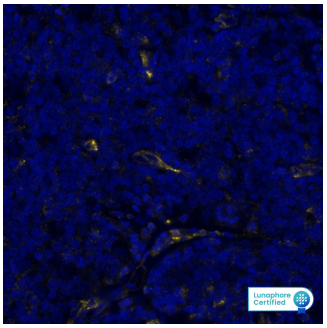
APPLICATIONS

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	Human placenta
Multiplex Immunofluorescence	5 µg/mL	Immersion fixed paraffin-embedded sections of human Hodgkin's Lymphoma and human Mantle Cell Lymphoma
Immunohistochemistry	0.5-10 µg/mL	Immersion fixed paraffin-embedded sections of human lymph node and tonsil

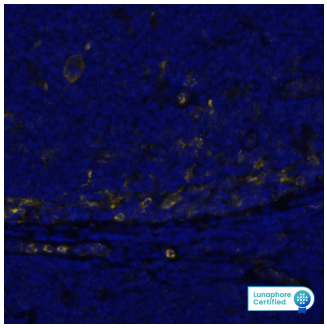
DATA

Multiplex Immunofluorescence



Detection of VISTA in Human Hodgkin's Lymphoma via seqIF™ staining on COMET™
VISTA was detected in immersion fixed paraffin-embedded sections of human Hodgkin's Lymphoma using Rabbit Anti-Human VISTA, Monoclonal Antibody (Catalog # MAB11712) at 5ug/mL at 27° Celsius for 2 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9; Epredia Catalog # TA-999-DHBH). Tissue was stained using the Alexa Fluor™ Plus 647 Goat anti-Rabbit IgG Secondary Antibody at 1:200 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR647RB) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the membrane and cytoplasm. Protocol available in COMET™ Panel Builder.

Multiplex Immunofluorescence



Detection of VISTA in Human Mantle Cell Lymphoma via seqIF™ staining on COMET™
VISTA was detected in immersion fixed paraffin-embedded sections of human Mantle Cell Lymphoma using Rabbit Anti-Human VISTA, Monoclonal Antibody (Catalog # MAB11712) at 5ug/mL at 27° Celsius for 2 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9; Epredia Catalog # TA-999-DHBH). Tissue was stained using the Alexa Fluor™ Plus 647 Goat anti-Rabbit IgG Secondary Antibody at 1:200 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR647RB) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the membrane and cytoplasm. Protocol available in COMET™ Panel Builder.

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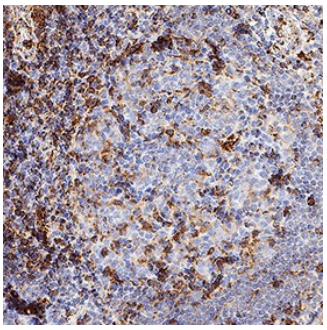
Human VISTA/B7-H5/PD-1H Antibody

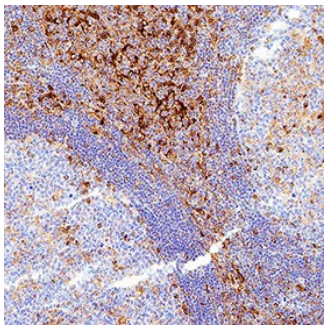
Recombinant Monoclonal Rabbit IgG Clone # 3180A
Catalog Number: MAB11712

Immunohistochemistry



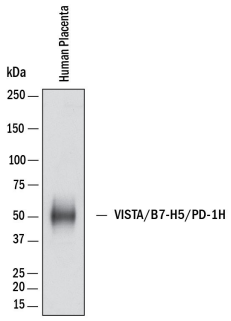
Detection of VISTA/B7-H5/PD-1H in Human Lymph Node. VISTA/B7-H5/PD-1H was detected in immersion fixed paraffin-embedded sections of human lymph node using Rabbit Anti-Human VISTA/B7-H5/PD-1H Monoclonal Antibody (Catalog # MAB11712) at 0.5 µg/ml for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003) or the HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using VisUCyte Antigen Retrieval Reagent-Basic (Catalog # VCTS021). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to the cell surface. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Immunohistochemistry



Detection of VISTA/B7-H5/PD-1H in Human Tonsil. VISTA/B7-H5/PD-1H was detected in immersion fixed paraffin-embedded sections of human tonsil using Rabbit Anti-Human VISTA/B7-H5/PD-1H Monoclonal Antibody (Catalog # MAB11712) at 0.5 µg/ml for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003) or the HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using VisUCyte Antigen Retrieval Reagent-Basic (Catalog # VCTS021). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to the cell surface. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Western Blot



Detection of Human VISTA/B7-H5/PD-1H by Western Blot. Western Blot shows lysates of human placenta. PVDF membrane was probed with 1 µg/ml of Rabbit Anti-Human VISTA/B7-H5/PD-1H Monoclonal Antibody (Catalog # MAB11712) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for VISTA/B7-H5/PD-1H at approximately 55 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

PREPARATION AND STORAGE

Reconstitution	Reconstitute lyophilized material at 0.2 mg/ml in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
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

Platelet Receptor Gi24, also known as VISTA (V-domain Ig suppressor of T cell activation), B7-H5, B7H5, Dies1 (Differentiation of ESC-1), SISP1 and C10orf54, 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 BMP-4, 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 human Gi24 contains a 162 aa extracellular region with one V-type Ig-like domain and a 96 aa cytoplasmic domain. Human Gi24 undergoes proteolytic cleavage by MT1-MMP, generating a soluble 30 kDa extracellular fragment plus a 25-30 kDa membrane-bound fragment. Over aa 33-194, human Gi24 shares 70% and 67% aa identity with mouse and rat Gi24, respectively.

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