Human Osteoactivin/GPNMB Antibody

Monoclonal Mouse IgG_{2B} Clone # 1077401

Catalog Number: MAB11534

DESCRIPTION

Species Reactivity	Human
Specificity	Detects rhOsteoactivin in Direct ELISA.
Source	Monoclonal Mouse IgG_{2B} Clone # 1077401
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Mouse myeloma cell line NS0-derived recombinant human Osteoactivin Met1-Pro486 Accession # Q14956
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose.

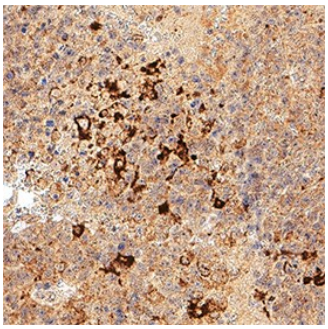
APPLICATIONS

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
Immunocytochemistry	3-25 µg/mL	fixed RT-4 human bladder carcinoma cell line and MOLT-4 human acute lymphoblastic leukemia cell line
Immunohistochemistry	3-25 µg/mL	Immersion fixed paraffin-embedded sections of human melanoma

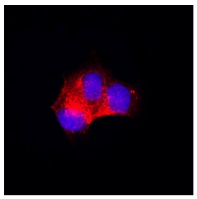
DATA

Immunohistochemistry

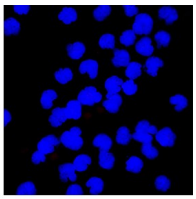


Detection of Osteoactivin/GPNMB in Human Melanoma. Osteoactivin/GPNMB was detected in immersion fixed paraffin-embedded sections of human melanoma using Mouse Anti-Human Osteoactivin/GPNMB Monoclonal Antibody (Catalog # MAB11534) at 0.5 µg/ml for 1 hour at room temperature followed by incubation with the HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007) or the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001). 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 cytoplasm and membrane. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Immunocytochemistry/ Immunofluorescence



RT-4 (Positive) cells



MOLT-4 (Negative) cells

Detection of Osteoactivin/GPNMB in RT-4 Human Cell Line and MOLT-4 Human Cell Line. Osteoactivin/GPNMB was detected in fixed RT-4 human bladder carcinoma cell line and MOLT-4 human acute lymphoblastic leukemia cell line using Mouse Anti-Human Osteoactivin/GPNMB Monoclonal Antibody (Catalog # MAB11534) at 8 µg/ml for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to the cytoplasm and membrane. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

PREPARATION AND STORAGE

Reconstitution	Reconstitute lyophilized material at 0.2mg/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.

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BACKGROUND

Osteoactivin (also GPNMB and DC-HIL) is a variably glycosylated 75-125 kDa member of the NMB/pMEL-17 family of molecules. It is found in multiple subcellular sites, but is most often associated with the endosomal/lysosomal compartment (1-3). Human Osteoactivin is a 560 amino acid (aa) type I transmembrane protein. Its precursor contains a 21 aa signal sequence, a 465 aa luminal/extracellular domain, a 21 aa transmembrane segment and a 53 aa cytoplasmic tail (4, 5). The luminal region contains an N-terminal heparin-binding motif (aa 23-26), multiple glycosylation sites, an RGD motif (aa 64-66) and an 88 aa PKD domain (aa 240-327). The intracellular tail has an ITAM (Y-x-x-I) and lysosomal targeting (L-L) motif (4, 5). The extracellular/luminal region shares 74% and 77% aa identity with the equivalent regions in mouse and canine, respectively. Multiple isoforms would appear to exist. There is one alternate splice form known that shows a 12 aa insert between aa 339-340 (6). An addtional 206 aa isoform shows a mutation at position # 181 that results in a 26 aa substitution for the C-terminal 380 amino acids (7, 8). This has the potential to be soluble and may represent a counterpart to a secreted isoform of rat Osteoactivin (9). Cells known to express Osteoactivin include macrophages/Kupffer cells, fibroblasts, osteoblasts, myeloid dendritic cells, retinal pigment epithelial cells and melanocytes, plus fetal chondrocytes and stratum basale keratinocytes (3-5, 10-12). In mice, Osteoactivin is reported to bind to heparan sulfate-proteoglycan, possibly on the surface of endothelial cells and may also interact with integrins (13). It also appears to act as an inflammatory suppressor gene, as its expression downregulates the macrophage inflammatory response by inhibiting IL-6 and IL-12 p40 production (3).

References:

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