

DESCRIPTION	
Species Reactivity	Human
Specificity	Detects human Osteoactivin in ELISAs and Western blots. In sandwich immunoassays, less than 2% cross-reactivity with recombinant mouse Osteoactivin is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human Osteoactivin isoform b Lys23-Asn486 Accession # NP_002501
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 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	0.5 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Human Osteoactivin/GPNMB Sandwich Immunoassay		Reagent
ELISA Capture	0.2-0.8 µg/mL	Human Osteoactivin/GPNMB Antibody (Catalog # AF2550)
ELISA Detection	0.1-0.4 µg/mL	Human Osteoactivin/GPNMB Biotinylated Antibody (Catalog # BAF2550)
Standard		Recombinant Human Osteoactivin/GPNMB Fc Chimera (Catalog # 2550-AC)

DATA

Western Blot

Detection of Human Osteoactivin/GPNMB by Western Blot. Western blot shows lysates of U-118-MG human glioblastoma/astrocytoma cell line and T98G human glioblastoma cell line. PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human Osteoactivin/GPNMB Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2550) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). Specific bands were detected for Osteoactivin/GPNMB at approximately 95 and 120 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry

Osteoactivin/GPNMB in Human Liver. Osteoactivin/GPNMB was detected in immersion fixed paraffin-embedded sections of human liver using Goat Anti-Human Osteoactivin/GPNMB Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2550) at 3 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). Specific staining was localized to Kupffer cells. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 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

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-l) 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 additional 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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