Human/Mouse/Rat GPR64 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF7977

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat GPR64 in direct ELISA and Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Chinese hamster ovary cell line CHO-derived recombinant human GPR64 Leu38-Asn64, Glu68-Thr553 Accession # NP_001073328
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.

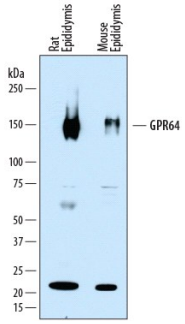
APPLICATIONS

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	2 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunohistochemistry	5-15 µg/mL	See Below

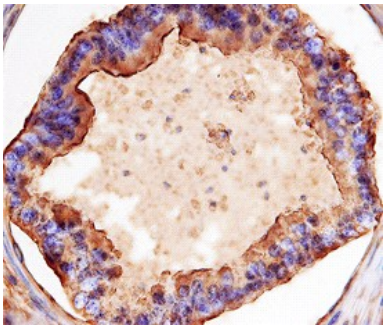
DATA

Western Blot



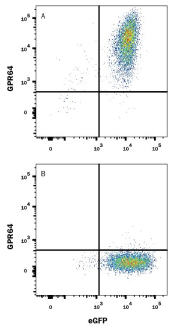
Detection of Rat and Mouse GPR64 by Western Blot. Western blot shows lysates of rat epididymis tissue and mouse epididymis tissue. PVDF membrane was probed with 2 µg/mL of Sheep Anti-Human/Mouse/Rat GPR64 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7977) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for GPR64 at approximately 150 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



GPR64 in Human Epididymus. GPR64 was detected in immersion fixed paraffin-embedded sections of human epididymus using Sheep Anti-Human/Mouse/Rat GPR64 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7977) at 3 µg/mL overnight at 4 °C. 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 the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to plasma membranes of epithelial cells. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Flow Cytometry



Detection of GPR64 in HEK293 Human Cell Line Transfected with Human GPR64 and eGFP by Flow Cytometry. HEK293 human embryonic kidney cell line transfected with (A) Human GPR64 or (B) Human GPR75 and eGFP was stained with Sheep Anti-Human GPR64 Affinity-Purified Polyclonal Antibody (Catalog # AF7977) 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.

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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.** ● 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

GPR64 (G-Protein coupled Receptor 64; also HE6) is a 110 kDa (predicted) member of the B class of GCPRs. Within this class GPR64 belongs to a Large N-termini family-B 7-transmembrane (LNB-7TM) subclass of receptors (also known as adhesion-GCPRs). GPR64 has restricted expression, being found in stereocilia cell membranes of epididymal caput epithelial cells and, to a limited extent, on osteoblasts. The function of GPR64 is somewhat unclear, but in the epididymis, it may be involved in fluid transport. Mature human GPR64 is a 980 amino acid (aa) 7-TM glycoprotein (SwissProt Q8IZP9). It contains an extended extracellular N-teminus (aa 38-627), seven consecutive TM segments (aa 628-878) and a C-terminal cytoplasmic tail (aa 879-1010). The extended extracellular region possesses a juxtamembrane GPS domain (aa 567-618) that serves as a proteolytic cleavage site. Enzymatic activity here generates a 180 kDa soluble form that stays associated with a 40 kDa membrane-embedded fragment. Notably, isolation of the membrane fragment gives rise to oligomers that run at > 250 kDa in SDS-PAGE. There are multiple splice variants. The one used for immunization to generate this antibody contains a deletion of aa 88-101 (RefSeq NP_001073328). Four other splice forms show single block deletions of aa 65-67, 51-66, 52-75, and 906-956, respectively. Three others possesses aa substitutions; a 20 aa block for aa 52-101, and a common 12 aa block that can substitute for either aa 68-101 or aa 52-101. Over aa 38-64 and 68-553 of RefSeq NP_001073328, human and mouse GPR64 share 69% aa sequence identity.

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