Recombinant Human/Mouse Wnt-5a

Catalog Number: 645-WN

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Wnt-5a protein Gln38-Lys380 Accession # P22725
N-terminal Sequence Analysis	Asn44 & No results obtained: Gln38 predicted
Predicted Molecular Mass	38 kDa

SPECIFICATIONS

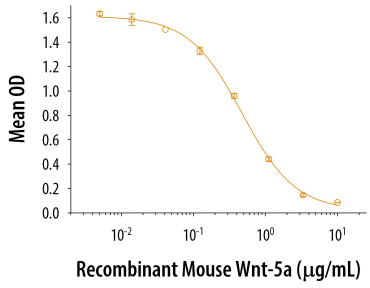
SDS-PAGE	45 kDa, reducing conditions
Activity	Measured by its ability to inhibit Wnt-3a-induced alkaline phosphatase production by MC3T3-E1 mouse preosteoblast cells. The ED_{50} for this effect is 0.1-0.5 µg/mL, in the presence of 5 ng/mL rmWnt-3a. **Optimal concentrations should be determined by each laboratory for each application.**
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>80%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS, EDTA and CHAPS with BSA as a carrier protein. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in sterile PBS containing at least 0.1% human or bovine serum albumin.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it 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. • 3 months, -20 to -70 °C under sterile conditions after reconstitution.

DATA

Bioactivity



Bioactivity of Mouse Wnt-5a
Recombinant mouse Wnt-5a (Catalog # 645-WN) inhibits Wnt-3a-induced alkaline phosphatase production in the MC3T3-E1 mouse preosteoblast cell line. The ED_{50} for this effect is 0.1-0.5 µg/mL in the presence of 5 ng/mL recombinant mouse Wnt-3a (Catalog # 1324-WN).

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BACKGROUND

Wnt-5a is a 44-50 kDa member of the Wnt family of proteins (1-6). Based on its activity towards C57Mg mammary epithelium, it is classified as a nontransforming Wnt. Human Wnt-5a is synthesized as a 380 amino acid (aa) precursor that contains a 37 aa signal sequence, a 25 aa prosegment, and a 319 aa mature region (1, 2, 3). The mature region has 24 cysteine residues that form multiple intrachain disulfide bonds, plus four N-linked glycosylation sites that are utilized for proper secretion (3, 5, 7). There is also a palmitate adduct at Cys104 that is essential for activity, and a potential palmitoleic acid modification at Ser244 that may also contribute to secretion (7-9). One alternative start site is reported at Met16. Over aa 38-380, human and mouse Wnt-5a are identical in amino acid sequence (1, 10).

Cells known to express Wnt-5a include brainstem astrocytes (11), mammary epithelium (12), CD34+ primitive progenitor stem cells (13), chondrocytes (14), $CD34^-$ pericytes and vascular smooth muscle cells (15), plus mesenchymal cells at various sites (16, 17). There are multiple receptors for Wnt-5a. These include Fzd-1, -2, -3, -4, -5, and -7 (3, 18-22), Ror2 (3), LRP6 (23), Ryk (24) and sFRP1 (25). All these molecules function within the context of a larger number of “co-factors” that regulate signaling by the Wnts. Initially, it was suggested that there were three pathways for Wnt signaling; a β-catenin-mediated canonical pathway, and two noncanonical pathways described as the Wnt/JNK (PCP) pathway and the Wnt/Ca^{++} pathway (26, 27). And it was assumed that various Wnts could be accommodated by these classifications. At present, it is now recognized that individual Wnts, through various combinations of receptor complex subunits, can have diverse effects, perhaps even within the same cell (3, 6, 27). Further complexity is introduced by the fact that Xenopus Wnt-5a and Wnt-11 are known to form bioactive heterodimers following Tyr sulfation (28). Thus, predicting the activity of Wnt-5a, or any other Wnt, on any cell type will require substantial insight into the interaction between all the extracellular, cell surface and intracellular components of the Wnt signaling system.

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