Recombinant Mouse Wnt-9a

Catalog Number: 8148-WN/CF

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived mouse Wnt-9a protein Tyr30-Gly365 Accession # Q8R5M2
N-terminal Sequence Analysis	Tyr30
Predicted Molecular Mass	37 kDa

SPECIFICATIONS

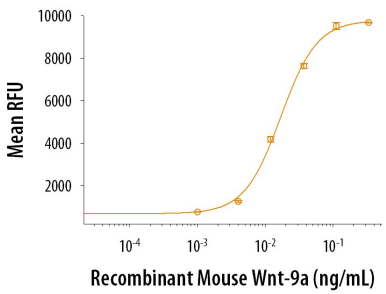
SDS-PAGE	36-43 kDa, reducing conditions
Activity	Measured by its ability to activate Wnt induced TCF reporter activity in HEK293 human embryonic kidney cells expressing human Frizzled-4 and human LRP-5. The ED_{50} for this effect is 8-40 ng/mL. Measured in a cell proliferation/survival assay using C3H10T1/2 mouse embryonic fibroblast cells. The ED_{50} for this effect is 60-300 ng/mL
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, 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. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 250 µg/mL in PBS.
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



Wnt-9a Activates Wnt Signaling in HEK293 Cells. Recombinant Mouse Wnt-9a (Catalog # 8148-WN/CF) activates TCF reporter activity in HEK293 human embryonic kidney cells expressing human Frizzled-4 and human LRP-5. The ED_{50} for this effect is 8-40 ng/mL.

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USA | TEL: 800.343.7475 Canada | TEL: 855.668.8722 Europe | Middle East | Africa TEL: +44.0.1235.529449
China | info.cn@bio-techne.com TEL: 400.821.3475

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BACKGROUND

Wnt-9a (Wingless integration region 9a; also Wnt-14) is a cysteine-rich member of the Wnt family of secreted glycoproteins (1-4). This family is rather large at 19 members, and although it is most often associated with embryogenesis, the Wnt family is now known to be also important in a number of related processes, including angiogenesis, wound healing, tissue remodeling, and cancer (2, 5-8). Wnt-9a represents a paralog (common ancestor; different function) to Wnt-9b/Wnt-14b which, in the mouse, are found on the same chromosome (2, 4). The mouse Wnt-9a precursor is 365 amino acids (aa) in length. It contains a 29 aa signal sequence coupled to a 336 aa mature region (aa 30-365) that is characterized by the presence of 24 Cys residues, two palmitoylation sites (Cys93 and Ser221), and one presumably utilized glycosylation site at Asn103 (4, 9). The glycosylation and palmitoylation appear essential for secretion and activity. Based on studies with other Wnts, the palmitic acid at Cys93 is essential for frizzled/FZD4, 7 and 9, WIF-1 activation, while Ser221 is necessary for secretion (10). Although the exact mechanism(s) for secretion is unclear, it would appear that Wnt-9a initially binds to a select GPCR (Evi) in the Golgi. As a complex, it is transported to the plasma membrane in where it is inserted, internalized, and budded-off in a microvesicle that returns to the cell membrane for release as an exosome (11). Over the mature region, mouse Wnt-9a is identical in aa sequence to rat Wnt-9a and shares 98% aa sequence identity with human Wnt-9a (12). With respect to its paralog (Wnt-9b), mouse Wnt-9a and -9b share only 65% aa sequence identity in the mature region (4, 13). Cells known to express Wnt-9a, either inducibly or constitutively, include skeletal muscle (14, 15), limb-bud mesenchyme (16), mammary epithelium (17, 18), embryonic hepatic stellate/Ito and sinusoidal endothelial cells (19), and cranial osteoblasts (20). There are an extraordinary number of receptors/binding proteins that have been identified for members of the Wnt family (1, 21, 22). To date, Wnt-9a has been reported to bind to Fzd4, 7 and 9 (19), WIF-1 (16), and MuSK in conjunction with LRP4 (15). Functionally, Wnt-9a is best known for its participation in joint formation (but not initiation). During the formation of cartilaginous tissue, Wnt-9a contributes to the process of cavitation where chondrogenic tissue undergoes apoptosis and remodeling to create a functional space (interzone; precursor to a joint) (23). Elsewhere in the embryo, Wnt-9a is posited to induce hepatocyte proliferation and promote the generation of glycogen through enzyme activity regulation (19). In addition, Wnt-9a is reported to induce acetylcholine receptor clustering in newly formed myotubes (15).

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