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RECOMBINANT MOUSE WNT-5A

CATALOG NUMBER:	GF146	**QUANTITY:**	100 µL
LOT NUMBER:		**CONCENTRATION:**	80-100 ng/µL

DESCRIPTION:

Wnt5a, a member of the highly conserved Wnt protein family, is a 352 amino acid protein containing 23 conserved cysteines and contains a cysteine modified by palmitate which is essential for receptor binding and biological activity. The Wnt5a amino acid sequence also contains an N-terminal signal peptide which means the protein is secreted into the medium when expressed in cell culture.

BACKGROUND:

Wnt ligands bind to cell surface co-receptors consisting of a Frizzled protein and lipoprotein receptor related protein (5/6). Wnt 5a belongs to the non-canonical class of Wnt proteins which are independent of or inhibit β-catenin signalling. Wnt5a when bound to its receptor triggers an intracellular Ca release which activates PKC and CaMK11 which in turn activates TAK1 MAPkinase-kinase and nemo-like(NLK) kinase. NLK phosphorylates Lef1/TCF protein complex inhibiting the formation of the β-catenin Lef1/TCF DNA complex. In contrast Wnt3a signalling stabilises β-catenin increasing the formation of the β-catenin Lef1/TCF DNA complex.

Wnt signalling has been implicated in the control of differentiation of stem cells. The Wnts have also been shown to have putative roles in the regulation of adult stem cells. Recently injection of embryonic stem cells into hearts has been shown to correct cardiac defects. This occurs through the secretion of factors from stem cells which correct gene defects in neighbouring cells. Wnt5a, a short term factor secreted by the stem cells, has been shown to be critical for this process (Fraidenraich and Benzra, 2006).

SOURCE:

The Wnt5a supplied has been purified from conditioned media using blue-sepharose and gel filtration.

PURITY:

Not determined. Endotoxin level is less than 0.2 ng per µg of Wnt5a.

ACTIVITY:

The biological activity of Wnt5a has been demonstrated by its ability to antagonize the induction of alkaline phosphatase synthesis by Wnt3a in the presence of BMP4 by C2C12 osteoblastic cells. The EC_{50} for this effect is typically 100 ng/mL.

Optimal concentrations should be determined for each application.

PRESENTATION:

Frozen liquid in PBS containing 1.0% Chaps.

STORAGE:

Maintain the frozen material at -20°C until expiration date as stated on the label.

- Upon receipt, thaw and aliquot into single-use aliquots and store at -20°C.
- Once a single-use aliquot has been thawed, DO NOT re-freeze.
- Store thawed material at 4°C for up to one month.

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PROTEIN HANDLING:

- This protein is extremely sticky and will stick to plasticware in the absence of Chaps detergent.
- When using this protein, if possible, dose it directly onto cells at the required dilution without pre-filtering.
- The toxicity of the Chaps in the storage buffer should be tested empirically on the cell line prior to using the Wnt5a protein.
- Generally, a minimum dilution of Wnt5a of 1:500 to 1:1000 on mammalian cells results in a final concentration of Chaps that is not toxic to the cells.
- If dosing on cells over a period of days/weeks, it is recommended that media containing Wnt5a is replenished daily, rather than dosing Wnt5a to existing media daily. This is because accumulation of Chaps is undesirable for cell viability.
- For SDS-Page and western analysis the protein should be diluted 1:1 with 10% SDS prior to the addition of Laemmli buffer.

REFERENCES:

Fraidenraich, D and Benzra, R. (2006). *Nature Clinical Practice Cardiovascular Medicine*. **3**: Suppl1 S14-17

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

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