APP549Ra01 10µg Active Wingless Type MMTV Integration Site Family, Member 5A (WNT5A) Organism Species: *Rattus norvegicus (Rat) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: lle62~Lys380 Tags: N-terminal His-tag Purity: >95% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose. Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 8.8 Predicted Molecular Mass: 39.5kDa Accurate Molecular Mass: 40kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

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Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

IIGAQPLCS QLAGLSQGQK KLCHLYQDHM QYIGEGAKTG IKECQYQFRH RRWNCSTVDN TSVFGRVMQI GSRETAFTYA VSAAGVVNAM SRACREGELS TCGCSRAARP KDLPRDWLWG GCGDNIDYGY RFAKEFVDAR ERERIHAKGS YESARILMNL HNNEAGRRTV YNLADVACKC HGVSGSCSLK TCWLQLADFR KVGDALKEKY DSAAAMRLNS RGKLVQVNSR FNSPTTQDLV YIDPSPDYCV RNESTGSLGT QGRLCNKTSE GMDGCELMCC GRGYDQFKTV QTERCHCKFH WCCYVKCKKC TEIVDQFVCK

[ACTIVITY]

Wingless Type MMTV Integration Site Family, Member 5A (WNT5A) is a ligand for members of the frizzled family of seven transmembrane receptors. Can activate or inhibit canonical Wnt signaling, depending on receptor context. Stimulates cell migration. Decreases proliferation, migration, invasiveness and clonogenicity of carcinoma cells and may act as a tumor suppressor. Besides, WNT Inhibitory Factor 1 (WIF1) has been identified as an interactor of WNT5A, thus a binding ELISA assay was conducted to detect the interaction of recombinant rat WNT5A and recombinant rat WIF1. Briefly, WNT5A were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to WIF1-coated microtiter wells and incubated for 2h at 37 $^\circ\!\!\mathbb{C}$. Wells were washed with PBST and incubated for 1h with anti-WNT5A pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 °C. Finally, add 50µL stop solution to the wells and read at 450nm immediately. The binding activity of WNT5A and WIF1 was shown in Figure 1, and this effect was in a dose dependent manner.

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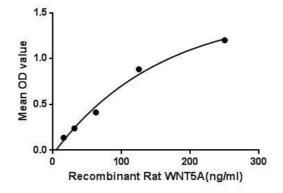


Figure 1. The binding activity of WNT5A with WIF1.

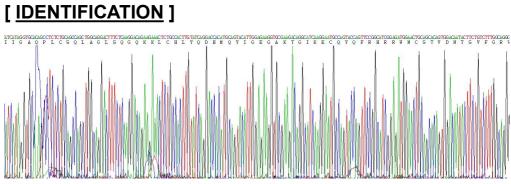
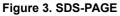


Figure 2. Gene Sequencing (extract)

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	18
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Sample: Active recombinant WNT5A, Rat

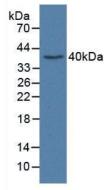


Figure 4. Western Blot Sample: Recombinant WNT5A, Rat; Antibody: Rabbit Anti-Rat WNT5A Ab (PAP549Ra01)

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.