APP121Hu01 10µg Active Wingless Type MMTV Integration Site Family, Member 16 (WNT16) Organism Species: *Homo sapiens* (Human) *Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Asn30~Lys365 Tags: N-terminal His and GST Tag Purity: >80% Buffer Formulation:PBS, pH7.4, containing 0.01% Sarcosyl, 5% Trehalose.

Original Concentration: 50µg/mL

Applications: Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 8.6

Predicted Molecular Mass: 70.0kDa

Accurate Molecular Mass: 70/60/40/38kDa as determined by SDS-PAGE reducing conditions.

Phenomenon explanation:

The possible reasons that the actual band size differs from the predicted are as follows:

- 1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
- 2. Relative charge: The composition of amino acids may affects the charge of the protein.
- 3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.

4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.

5. Polymerization of the target protein: Dimerization, multimerization etc.

[USAGE]

Cloud-Clone Corp. Reconstitute in ddH₂O to a concentration ≤0.1mg/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.

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.

[<u>SEQUENCE</u>]

N WMWLGIASFG VPEKLGCANL PLNSRQKELC KRKPYLLPSI REGARLGIQE CGSQFRHERW NCMITAAATT APMGASPLFG YELSSGTKET AFIYAVMAAG LVHSVTRSCS AGNMTECSCD TTLQNGGSAS EGWHWGGCSD DVQYGMWFSR KFLDFPIGNT TGKENKVLLA MNLHNNEAGR QAVAKLMSVD CRCHGVSGSC AVKTCWKTMS SFEKIGHLLK DKYENSIQIS DKTKRKMRRR EKDQRKIPIH KDDLLYVNKS PNYCVEDKKL GIPGTQGREC NRTSEGADGC NLLCCGRGYN THVVRHVERC ECKFIWCCYV RCRRCESMTD VHTCK

[ACTIVITY]

Wingless-type MMTV integration site family, member 16 is a protein that in humans is encoded by the WNT16 gene. The WNT gene family consists of structurally related genes that encode secreted signaling proteins. These proteins have been implicated in oncogenesis and in several developmental processes, including regulation of cell fate and patterning during embryogenesis. This gene is a member of the WNT gene family. It contains two transcript variants diverging at the 5' termini. These two variants are proposed to be the products of separate promoters and not to be splice variants from a single promoter. Besides, Tubulin Beta 3 (TUBb3) has been identified as an interactor of WNT16, thus a binding ELISA assay was conducted to detect the interaction of recombinant human WNT16 and recombinant human TUBb3. Briefly, WNT16 were diluted serially in

Cloud-Clone Corp. PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100µL were then transferred

to TUBb3-coated microtiter wells and incubated for 2h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-WNT16 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 WNT16 and TUBb3 was shown in Figure 1, and this effect was in a dose dependent manner.



Figure 1. The binding activity of WNT16 with TUBb3.



Figure 2. Gene Sequencing (extract)

Cloud-Clone Corp. kDa 70 44 33 26 22 18 14 10

Figure 3. SDS-PAGE

Sample: Active recombinant WNT16, Human

[IMPORTANT NOTE]

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