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APA303Hu01 100µg Active Active Galectin 3 (GAL3) Organism Species: *Homo sapiens* (Human) *Instruction manual*

FOR IN VITRO USE AND RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Met1~lle250

Tags: N-terminal His-tag

Purity: >98%

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: 8.6

Predicted Molecular Mass: 27.4kDa

Accurate Molecular Mass: 28kDa 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.

[<u>SEQUENCE</u>]

MADNFSLHDA LSGSGNPNPQ GWPGAWGNQP AGAGGYPGAS YPGAYPGQAP PGAYPGQAPP GAYPGAPGAY PGAPAPGVYP GPPSGPGAYP SSGQPSATGA YPATGPYGAP AGPLIVPYNL PLPGGVVPRM LITILGTVKP NANRIALDFQ RGNDVAFHFN PRFNENNRRV IVCNTKLDNN WGREERQSVF PFESGKPFKI QVLVEPDHFK VAVNDAHLLQ YNHRVKKLNE ISKLGISGDI DLTSASYTMI [ACTIVITY]

Galectin 3 (GAL3) is a member of the lectin family, of which 14 mammalian galectins have been identified. It is also a member of the beta-galactoside-binding protein family that plays an important role in cell-cell adhesion, cell-matrix interactions, macrophage activation, angiogenesis, metastasis, apoptosis. The protein also has been demonstrated to be involved in cancer, inflammation and fibrosis, heart disease, and stroke. GAL3 is expressed in the nucleus, cytoplasm, mitochondrion, cell surface, and extracellular space. Besides, Alpha-2-Heremans Schmid Glycoprotein (aHSG) has been identified as an interactor of GAL3, thus a binding ELISA assay was conducted to detect the interaction of recombinant human GAL3 and recombinant human aHSG. Briefly, GAL3 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to aHSG-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-GAL3 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 GAL3 and aHSG was shown in Figure 1, and this effect was in a dose dependent manner.

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Figure 1. The binding activity of GAL3 with aHSG.

GAL3 also can agglutinate red blood. In this case, we chose rabbit erythrocyte (RaE) to assay its ability of agglutination. A general procedure for hemagglutination assay (or haemagglutination assay; HA) is as follows, two-fold dilute the recombinant human GAL3 with 0.01M PBS (pH7.4), add 50uL a serial dilution of GAL3 to each well of a U or V- bottom shaped 96-well microtiter plate. The final well serves as a negative control with no GAL3, replace with 50uL 0.01M PBS. Then add 50uL 1% rabbit erythrocyte to each well and mixed gently. The plate is incubated for 1-2 hours at room temperature. The results are shown in Figure 2. It was obvious that the minimal effective concentration of GAL3 is 1.2ug/mL.



Α

В

(A) 1% RaE tread with 1.2ug/mL GAL3 for 2h;

(B) Negative control without GAL3.

Positive Negative

Figure 3. The hemagglutination assay of GAL3 in V- bottom shaped 96-well microtiter plate.

[IDENTIFICATION]

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Figure 4. Gene Sequencing (extract)

Figure 5. SDS-PAGE

Sample: Active recombinant GAL3, Human

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Figure 6. Western Blot Sample: Recombinant GAL3, Human; Antibody: Rabbit Anti-Human GAL3 Ab (PAA303Hu01)

[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.