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APA309Hu61 100µg Active Galectin 9 (GAL9) Organism Species: *Homo sapiens (Human) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression. Host: 293F cell Residues: Met1~Thr355 Tags: N-terminal His-tag **Purity: >95% Endotoxin Level:** <1.0EU per 1µg (determined by the LAL method). **Buffer Formulation:** PBS, pH7.4, containing 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: 9.3 Predicted Molecular Mass: 41.2kDa Accurate Molecular Mass: 50kDa 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.

[<u>USAGE</u>]

Reconstitute in 10mM PBS (pH7.6) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

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[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>]

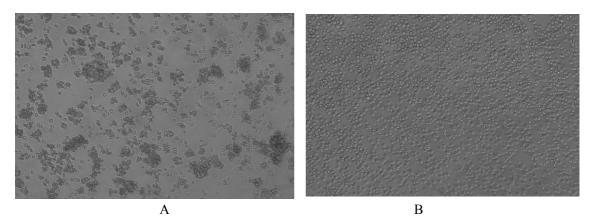
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TGFSGNDIAF HFNPRFEDGG YVVCNTRQNG SWGPEERKTH MPFQKGMPFD
LCFLVQSSDF KVMVNGILFV QYFHRVPFHR VDTISVNGSV QLSYISFQNP
RTVPVQPAFS TVPFSQPVCF PPRPRGRRQK PPGVWPANPA PITQTVIHTV
QSAPGQMFST PAIPPMMYPH PAYPMPFITT ILGGLYPSKS ILLSGTVLPS
AQRFHINLCS GNHIAFHLNP RFDENAVVRN TQIDNSWGSE ERSLPRKMPF
VRGQSFSVWI LCEAHCLKVA VDGQHLFEYY HRLRNLPTIN RLEVGGDIQL
THVQT
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[ACTIVITY]

Galectin 9 (GAL9) is a member of the β -galactoside-binding galectin family. Galectin-9 is found outside of cells and may be exported by non-classical pathways. Galectin 9 exhibits a variety of biological activities, the majority of which have focused on its immunomodulatory role toward lymphocytes, were it shows specific interactions with TIM-3, and can negatively regulate Th1 type immunity. It 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 GAL9 with 0.9% sodium chloride injection, add 50µL a serial dilution of GAL9 to each well of a U or V-bottom shaped 96-well microtiter plate. The final well serves as a negative control with no GAL9, replace with 50µL 0.9% sodium chloride injection. Then add 50µL 1% rabbit erythrocyte to each well and mixed

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gently. The plate is incubated for 3 hours at room temperature. The results were shown in Figure 1. It was obvious that the minimal effective concentration of GAL9 is $3.125 \ \mu g/mL$.





- (A) Rabbit erythrocyte agglutinated by recombinant human GAL9;
 - (B) Rabbit erythrocyte without recombinant human GAL9.

Negative

Positive



Figure 2. The hemagglutination assay of GAL9 in V- bottom shaped 96-well microtiter plate.

[IDENTIFICATION]

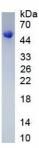


Figure 3. SDS-PAGE

Sample: Active recombinant GAL9, Human

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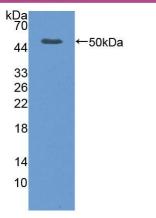


Figure 4. Western Blot Sample: Recombinant GAL9, Human; Antibody: Rabbit Anti-Human GAL9 Ab (PAA309Hu06)

[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.