APA309Hu01 50ug
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: Prokaryotic expression.
Host: *E. coli*
Residues: Met1~Thr323
Tags: N-terminal His-Tag.
Purity: >90%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.01% sarcosyl, 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: 8.3
Predicted Molecular Mass: 39.6kDa
Accurate Molecular Mass: 40kDa 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]

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 2-8ºC for one month.

Aliquot and store at -80ºC 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 37ºC 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.

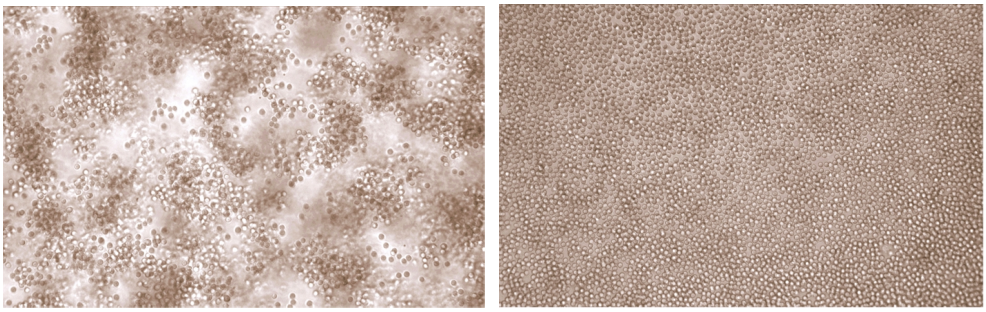
[SEQUENCE]

MAFSGSQAPY LSPAVPFSGT IQGGLQDGLQ ITVNGTVLSS SGTRFAVNFQ
TGFSGNDIAF HFNPRFEDGG YVVCNTRQNG SWGPEERKTH MPFQKGMPFD
LCFLVQSSDF KVMVNGILFV QYFHRVPFHR VDTISVNGSV QLSYISFQPP
GVWPANPAPI TQTVIHTVQS APGQMFSTPA IPPMMYPHPA YPMPFITTIL
GGLYPSKSIL LSGTVLPSAQ RFHINLCSGN HIAFHLNPRF DENAVVRNTQ
IDNSWGSEER SLPRKMPFVR GQSFSVWILC EAHCLKVAVD GQHLFEYYHR
LRNLPTINRL EVGGDIQLTH VQT

[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 0.01M PBS(pH7.4), 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 0.01M PBS. Then add 50μL 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 1. It was obvious that the minimal effective concentration of GAL9 is 1.5μg/mL.



A B

Figure 1. The hemagglutination of recombinant human GAL9
(A) Rabbit erythrocyte agglutinated by recombinant human GAL9;
(B) Rabbit erythrocyte without recombinant human GAL9.

Positive **Negative**

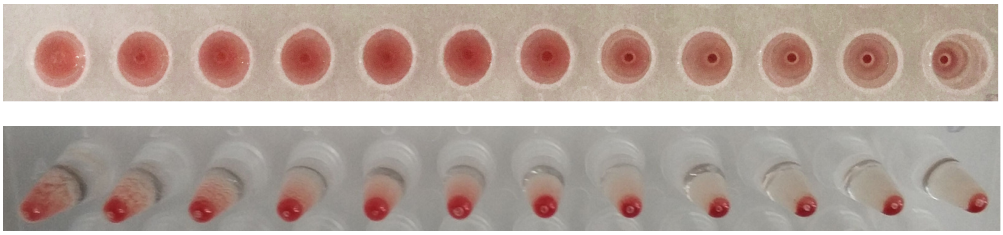


Figure 2. The hemagglutination assay of GAL9 in V- bottom shaped 96-well microtiter plate.

GAL9 also can induce cellular apoptosis. To test the effect of GAL9 on cell apoptosis, Jurkat cells were seeded into 96-well plates at a density of 5,000 cells/well with 2% serum standard RPMI 1640 including various concentrations of recombinant human GAL9. After incubated for 48 h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 20 μL of CCK-8 solution was added to each well of the plate, then the absorbance at 450 nm was measured using a microplate reader after incubating the plate for 4 h at 37℃. Proliferation of Jurkat cells after incubation with GAL9 for 48 h observed by inverted microscope was shown in Figure 3. Cell viability was assessed by CCK-8 assay after incubation with recombinant human GAL9 for 48 h. The result was shown in Figure 4. It was obvious that GAL9 significantly inhibit cell viability of Jurkat cells. The ED50 is 1.64 μg/mL.

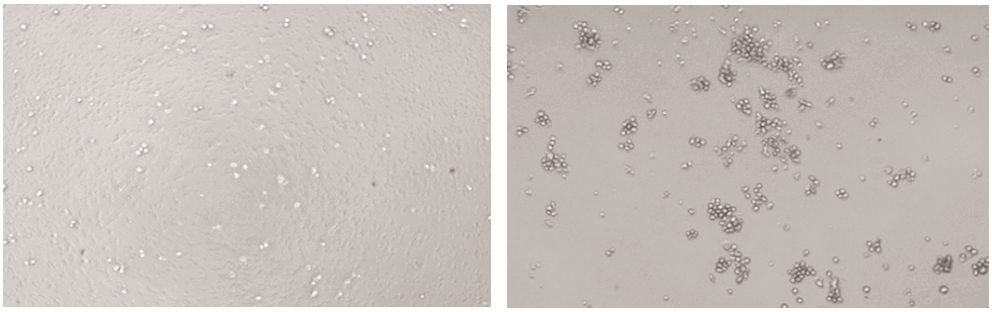


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Figure 3. Inhibition of Jurkat cells proliferation after stimulated with GAL9

(A) Jurkat cells cultured in RPMI 1640, stimulated with 2.5 μg/mL GAL9 for 48h;

(B) Unstimulated Jurkat cells cultured in RPMI 1640 for 48h.

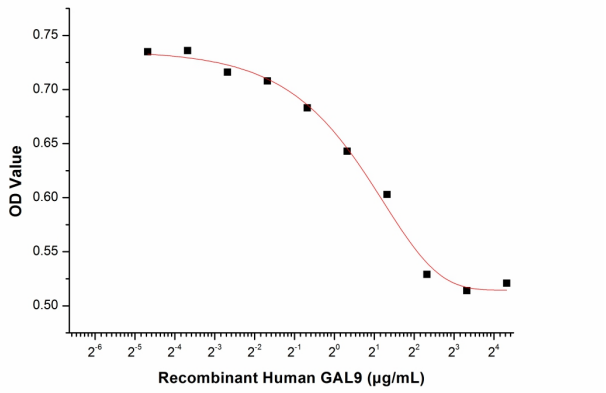

Figure 4. Inhibition of Jurkat cells proliferation after stimulated with GAL9.

[IDENTIFICATION]

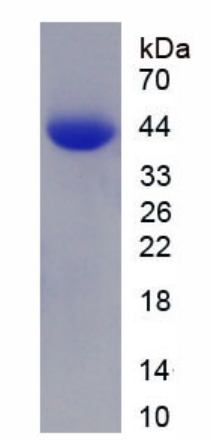

Figure 5. SDS-PAGE

Sample: Active recombinant GAL9, Human

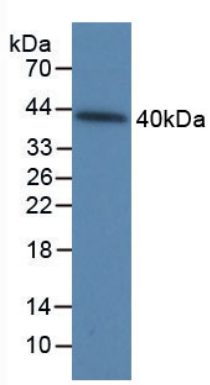

Figure 6. Western Blot

Sample: Recombinant GAL9, Human;

Antibody: Rabbit Anti-Human GAL9 Ab (PAA309Hu01)

[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