

APA204Hu01 100µg
Active Growth Arrest Specific Protein 6 (GAS6)
Organism Species: Homo sapiens (Human)
Instruction manual

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

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Leu136~Phe311

Tags: N-terminal His-tag

Purity: >92%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 1mM DTT, 0.01% sarcosyl, 5% trehalose, and Proclin300.

Predicted isoelectric point: 5.6

Predicted Molecular Mass: 20.4kDa

Accurate Molecular Mass: 24kDa as determined by SDS-PAGE reducing conditions.

Applications: Cell culture; Activity Assays; In vivo assays.

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

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 2-8°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.

[SEQUENCE]

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LMGNF FCLCKAGWGG  
RLCDKDVNEC SQENGGCLQI CHNKPFSFHC SCHSGFELSS DGRTCQDIDE  
CADSEACGEA RCKNLPGSYS CLCDEGFAYS SQEKACRDVD ECLQGRCEQV  
CVNSPGSYTC HCDGRGGLKL SQDMDTCEDI LPCVPFSVAK SVKSLYLGRM  
FSGTPVIRLR F
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[ACTIVITY]

Growth arrest-specific 6, also known as GAS6, is a gamma-carboxyglutamic acid (Gla) domain-containing protein thought to be involved in the stimulation of cell proliferation. It has been reported that both PC-3 and DU 145 human prostate cancer cell lines are stimulated to proliferate by Gas6, however, this proliferative response strictly correlates with the expression of the Axl receptor, being higher in DU 145 cells. To test the proliferative effect of Gas6, DU 145 cells were seeded into triplicate wells of 96-well plates at a density of 2,000 cells/well and allowed to attach overnight, then the medium was replaced with serum-free standard DMEM prior to the addition of various concentrations of GAS6. After incubated for 72h,

cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 μ L of CCK-8 solution was added to each well of the plate, then measure the absorbance at 450nm using a microplate reader after incubating the plate for 1-4 hours at 37 $^{\circ}$ C.

Cell proliferation of DU145 cells after incubation with GAS6 for 72h observed by inverted microscope was shown in Figure 1.

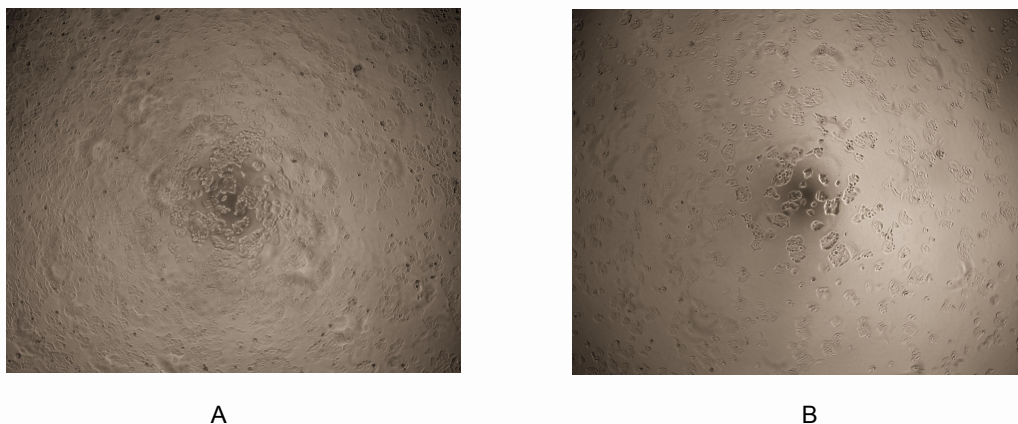


Figure 1. Cell proliferation of DU145 cells after stimulated with GAS6.

(A) DU145 cells cultured in serum-free DMEM, stimulated with 100ng/mL Gas6 for 72h;

(B) Unstimulated DU145 cells cultured in serum-free DMEM for 96h.

The dose-effect curve of GAS6 was shown in Figure 2. It was obvious that GAS6 significantly promoted cell proliferation of DU145 cells. The ED50 for this effect is typically 0.77~38.08ng/mL.

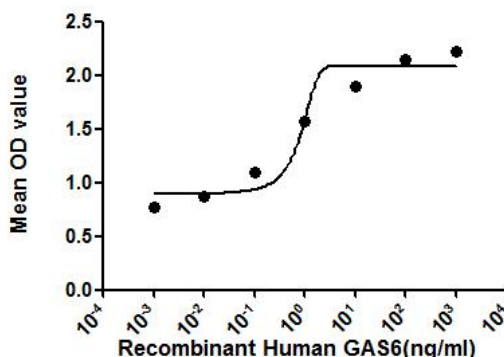


Figure 2. Cell proliferation of DU145 cells after stimulated with GAS6 detected by CCK8.

[**IDENTIFICATION**]

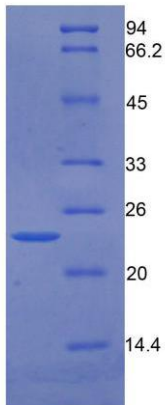


Figure 3. SDS-PAGE

Sample: Active recombinant GAS6, Human

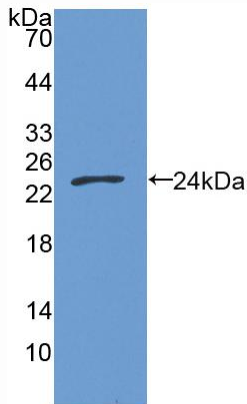


Figure 4. Western Blot

Sample: Recombinant GAS6, Human;

Antibody: Rabbit Anti-Human GAS6 Ab (PAA204Hu01)