APA045Ov01 100μg
Active Colony Stimulating Factor 2, Granulocyte Macrophage (GM-CSF)
Organism Species: *Ovis aries; Ovine (Sheep)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

12th Edition (Revised in Aug, 2016)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Ala18~Lys144
Tags: N-terminal His and GST Tag
Purity: >90%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5%Trehalose .
Original Concentration: 200μg/mL
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 5.1
Predicted Molecular Mass: 44.4kDa
Accurate Molecular Mass: 51kDa 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 10mM PBS (pH7.4) 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]

APTRQPSPVTRPWQHVDAIKEALSLLNDSTDTAAVMDETVEVVSEMFDSQEPTCLQTRLELYKQGLRGSLTSLTGSLTMMASHYKKHCPP
TQETSCETQIITFKSFKENLKDFLFIIPFDCWEPVQK

[ACTIVITY]

Granulocyte-macrophage colony-stimulating factor (GM-CSF), also known as colony-stimulating factor 2 (CSF2), is a monomeric glycoprotein that functions as a cytokine — it is a white blood cell growth factor. GM-CSF also has some effects on mature cells of the immune system. These include, for example, inhibiting neutrophil migration and causing an alteration of the receptors expressed on the cells surface. Besides,the proliferation of TF-1 cell line GM-CSF basic-dependent, thus, the activity of GM-CSF is usually measured by a cell proliferation assay using TF-1 cells. TF-1 cells were seeded into triplicate wells of 96-well plates at a density of 8,000 cells/well with 2% serum standard 1640 which contains various concentrations of recombinant sheep GM-CSF.

After incubated for 2 days, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 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 2-4 hours at 37 ℃. Proliferation of TF-1 cells after incubation with GM-CSF for 2 days observed by inverted microscope was shown in Figure 1. Cell viability was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with recombinant sheep GM-CSF for 2 days. The result was shown in Figure 2. It was obvious that GM-CSF significantly increased cell viability of TF-1 cells. The ED50 is 13.9 ng/ml.

Figure 1. Cell proliferation of TF-1 cells after stimulated with GM-CSF.

(A) TF-1 cells cultured in 1640, stimulated with 1ug/ml GM-CSF for 2 days;
(B) Unstimulated TF-1 cells cultured in 1640 for 2 days.

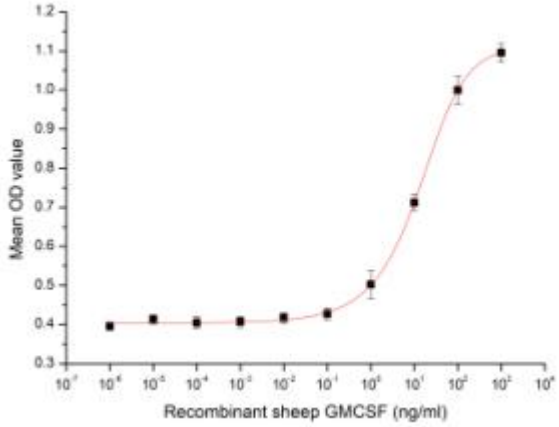

Figure 2. Cell proliferation of TF-1 cells after stimulated with GM-CSF.

[IDENTIFICATION]

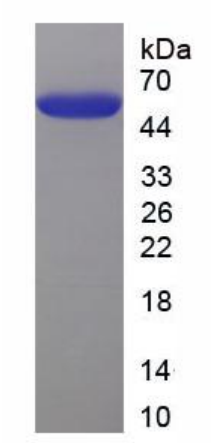

Figure 3. SDS-PAGE

Sample: Active recombinant GM-CSF, Sheep

[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.