APA045Hu61 50μg
Active Colony Stimulating Factor 2, Granulocyte Macrophage (GM-CSF)
Organism Species: Homo sapiens (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Eukaryotic expression.
Host: 293F cell
Residues: Ala18~Glu144
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.
Original Concentration: 2000μg/mL
Applications: Cell culture; Activity Assays; In vivo assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 5.2
Predicted Molecular Mass: 16.1kDa
Accurate Molecular Mass: 19-26kDa 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.

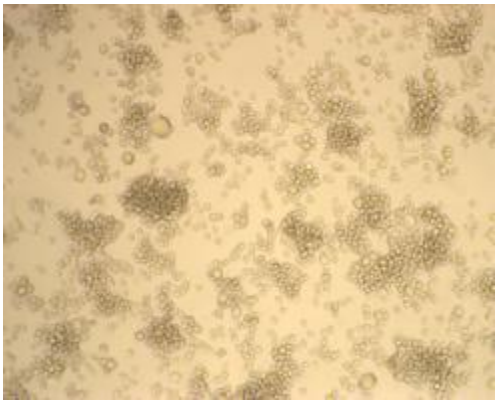
[SEQUENCE]

APA RSPSPSTQPW EHVNAIQEAR RLLNLSRDTA
AEMNETVEVI SEMFDLQEPT CLQTRLELYK QGLRGSLTKL KGPLTMMASH
YKQHCPPTPE TSCATQIITF ESFKENLKDF LLVIPFDCWE PVQE

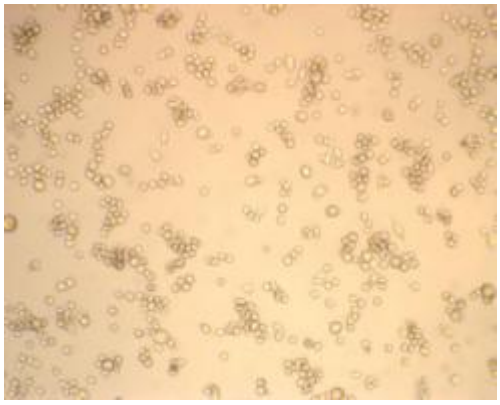
[ACTIVITY]

Mechanism: GM-CSF is a monomeric glycoprotein that functions as a cytokine, which stimulates stem cells to produce granulocytes (neutrophils, eosinophils, and basophils) and monocytes. And it's known that TF-1 is a cell line of immature erythroid origin that completely depends on interleukin 3 (IL-3) or granulocyte-macrophage colony-stimulating factor (GM-CSF) for long term growth. So the activity of GM-CSF is usually measured by a cell proliferation assay using TF-1 cells. Briefly, TF-1 cells were incubated in 96-well microplates in the presence or absence of various concentrations of rhGM-CSF, then cells were observed by inverted microscope everyday and cell proliferation was examined with CCK-8 (Cell Counting Kit-8) assay. As reported, GM-CSF was also able to induce differentiation of human monoblastic leukemia cell line U937. Therefore, U937 cells were incubated with various concentrations of rhGM-CSF (1ng/mL, 10ng/mL, 100ng/mL), then cells were observed by inverted microscope everyday. Result 1: After incubation with GM-CSF (10ng/mL) for 3 days, significant cell proliferation was observed in TF-1 cells compared with the control group (Figure 1).The ED50

is 0.74ng/ml.



A



B

Figure 1. Effect of GM-CSF on TF-1 cells.

(A) TF-1 cells cultured in RPMI 1640, stimulated with GM-CSF (2ng/mL);

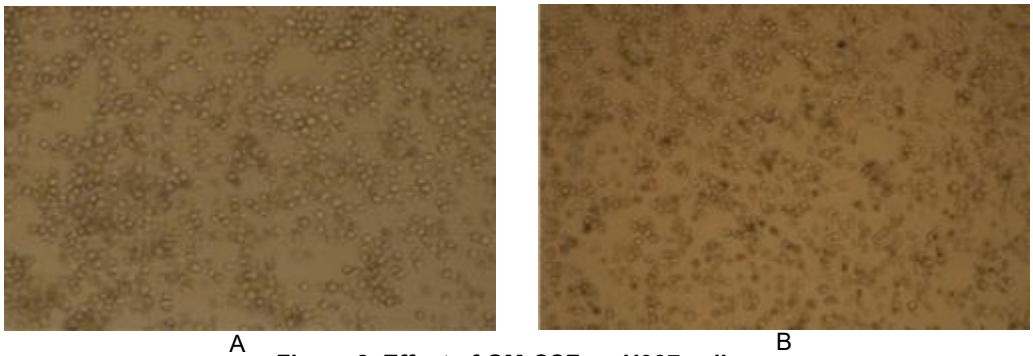
(B) Unstimulated TF-1 cells cultured in RPMI 1640.

Result 2: After incubation with GM-CSF (1ng/mL, 10ng/mL, 100ng/mL) for 3 days, significant cell proliferation was detected by CCK-8 assay in TF-1 cells compared with the control group (Table 1).

Table 1. Effect of GM-CSF on TF-1 cells by CCK-8

Sample (TF-1 cells)	OD1	OD2
stimulated with GM-CSF (1ng/mL)	1.226	1.167
stimulated with GM-CSF (10ng/mL)	1.324	1.401
stimulated with GM-CSF (100ng/mL)	1.367	1.416
unstimulated (control)	0.851	0.941

Result 3: After incubation with GM-CSF (10ng/mL) for 5 days, cell morphological change was observed in some U937 cells, which showed a morphological characteristics of dendritic cells compared with the control group (Figure 2).



A B

Figure 2. Effect of GM-CSF on U937 cells.

(A) U937 cells cultured in RPMI 1640, stimulated with GM-CSF (10ng/mL);

(B) Unstimulated U937 cells cultured in RPMI 1640.

[IDENTIFICATION]

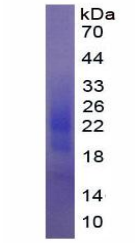


Figure 3. SDS-PAGE

Sample: Active recombinant GMCSF, Human

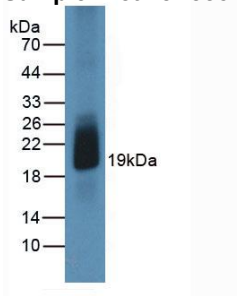


Figure 4. Western Blot

Sample: Recombinant GMCSF, Human;

Antibody: Rabbit Anti-Human GMCSF Ab (PAA045Hu06)

[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.