APA042Ra61 100μg
Active Colony Stimulating Factor 3, Granulocyte (GCSF)
Organism Species: *Rattus norvegicus (Rat)*
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: Ile22~Leu198
Tags: N-terminal His-tag
Purity: >90%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 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: 7.3
Predicted Molecular Mass: 21.1kDa
Accurate Molecular Mass: 25&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]

IPLLTVSSL PPSLPLPRSF LLKSLEQVRK
IQARNTELLE QLCATYKLCH PEELVLFGHS LGIPKASLSS CSSQALQQTK
CLSQLHSGLF LYQGLLQALA GISSELAPTL DMLHLDVDNF ATTIWQQMES
LGVAPTVQPT QSTMPIFTSA FQRRAGGVLV TSYLQSFLET AHHALHHL

[ACTIVITY]

G-CSF is a pleiotropic cytokine best known for its specific effects on the proliferation, differentiation, and activation of hematopoietic cells of the neutrophilic granulocyte lineage. It is produced mainly by monocytes and macrophages upon activation by endotoxin, TNF-alpha and IFN-gamma. In addition, various carcinoma cell lines and myeloblastic leukemia cells can express G-CSF constitutively. In vitro, G-CSF stimulates growth, differentiation and functions of cells from the neutrophil lineage. Consistent with its in vitro functions, G-CSF has been found to play important roles in defense against infection, in inflammation and repair, and in the maintenance of steady state hematopoiesis. The activity of G-CSF is usually measured by a cell proliferation assay using M-NFS-60 mouse myelogenous leukemia lymphoblast cells.

H-M-NFS-60 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 rat G-CSF. After incubated for 3 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 M-NFS-60 cells after incubation with G-CSF for 3 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 rat G-CSF for 3 days. The result was shown in Figure 2. It was obvious that G-CSF significantly increased cell viability of M-NFS-60 cells. The EC50 is 0.79-1.26 pg/ml

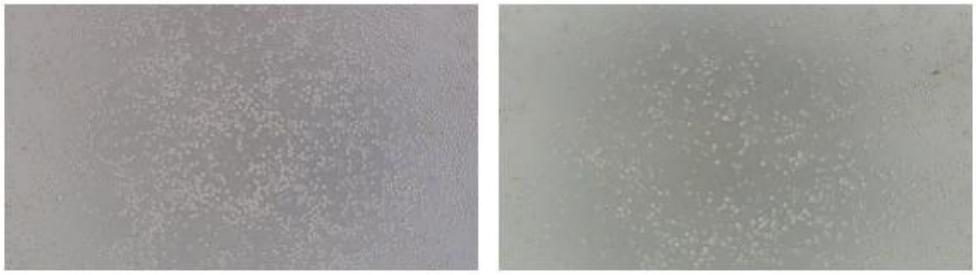

Figure 1. Cell proliferation of M-NFS-60 cells after stimulated with G-CSF.
(A) M-NFS-60 cells cultured in 1640, stimulated with 1 pg/ml G-CSF for 3 days;
(B) Unstimulated M-NFS-60 cells cultured in 1640 for 3 days.

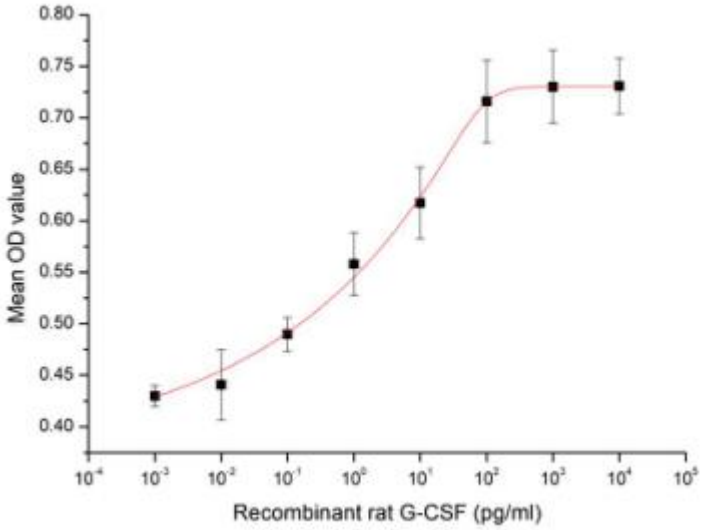

Figure 2. Cell proliferation of M-NFS-60 cells after stimulated with G-CSF.

[IDENTIFICATION]

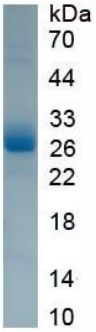

Figure 3. SDS-PAGE

Sample: Active recombinant GCSF, Rat

[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.