AEK0010

# Human G-CSF High Sensitivity

# ELISA Kit

Quantitative detection of human granulocyte colony stimulating factor (G-CSF) concentration in serum, plasma and cell culture supernatant.

This product is only used for scientific research. It is not a diagnostic reagent and cannot be used for clinical diagnosis.

## 1. Product introduction

### 1. Background introduction

Granulocyte colony stimulating factor (G-CSF) is a

glycoprotein that stimulates the bone marrow to produce granulocytes and stem cells and release them into the blood. Functionally, it is a cytokine and hormone, a colony stimulating factor, produced by endothelial cells, macrophages and many other immune cells. G-CSF stimulates the survival, proliferation, differentiation and function of neutrophil precursors and mature neutrophils.

In oncology and hematology, recombinant G-CSF is used to accelerate the recovery of neutropenia caused by chemotherapy in cancer patients. Before white blood cell removal, G-CSF can also be used to increase the number of hematopoietic stem cells in the blood of donors for hematopoietic stem cell transplantation.

2. Detection principle

This kit uses double antibody sandwich enzyme-linked immunosorbent detection technology. The specific anti-human G-CSF antibody is pre-coated on a high-affinity ELISA plate. The standard substance and the sample to be tested are added to the wells of the microtiter plate, and after incubation, the G-CSF present in the sample binds to the solid-phase antibody. After washing to remove unbound

material, add biotinylated detection antibody and incubate. Wash to remove unbound biotinylated antibody, and add horseradish peroxidase-labeled streptavidin (Streptavidin-HRP). After washing, add signal enhancer to incubate, after washing to remove unbound substances, add Streptavidin-HRP again. After washing, add TMB, a chromogenic substrate, to avoid light for color development. The intensity of the color response is directly proportional to the concentration of G-CSF in the sample. Add stop solution to stop the reaction, and measure the absorbance value at 450 nm wavelength (reference wavelength 570-630 nm).

3. Limitations of kit detection

1) Please use within the validity period marked on this kit.

2) The reagents of the kit cannot be mixed with reagents of other batch numbers or reagents from other sources.

3) Any changes in standard dilution, operators, pipetting techniques, washing techniques, incubation temperature, and kit storage time will affect the binding reaction.

4) This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

2. Basic information

1. Materials provided by the kit

| Component | number | EK169HS - 24 | EK169HS - 48 | EK169HS - 96 |
|---|---|---|---|---|
| Pre-coated ELISA plate | EK169HSP | 24T | 48T | 96T |
| Standard | EK169HSS | 1 vial | 1 vial | 2 vials |
| Detection antibody | EK169HSD | 1 vial | 1 vial | 1 vial |
| Standard Diluent | E0260 | 5 ml | 5 ml | 5 ml |
| Horseradish peroxidase | E0290 | 1 vial | 1 vial | 2 vials |
| Signal enhancer concentrate | E0270 | 1 vial | 1 vial | 1 vial |
| Signal Enhancer Diluent | E0320 | 12 ml | 12 ml | 12 ml |
| 10× Detection Buffer | E0310 | 5 ml | 5 ml | 5 ml |
| Chromogenic substrate TMB | E0230 | 3 ml | 6 ml | 11 ml |
| Stop solution | E0300 | 11 ml | 11 ml | 11 ml |
| 20× lotion | E0281 | 50 ml | 50 ml | 50 ml |
| Sealing film | E0200 | 5 | 10 | 10 |

1 vial 2 vials

Detection

antibody

EK169HSD 1 vial

1 vial 1 vial

Standard Diluent

E0260 5 ml 5 ml 5

ml

Horseradish peroxidase

Tagged streptavidin E0290 1 vial 1 vial 2 vials

E0270 1 vial 1 vial 1 vial

E0320 12 ml 12 ml 12 ml

E0310 5 ml 5 ml 5 ml

TMB E0230 3 ml 6 ml 11 ml

Stop solution E0300 11 ml 11 ml 11 ml

20× lotion E0281 50 ml 50 ml 50 ml

E0200 5 10 10

2. Materials and equipment not provided

1) A microplate reader capable of detecting absorbance at 450 nm, with a reference wavelength of 570 nm or 630 nm

2) Pipette and pipette tip, sample slot

3) Prepare test tubes, centrifuge tubes, graduated cylinders, etc. for reagents

4) Distilled water or deionized water

5) Vortex oscillator, microplate oscillator

3. Storage

The kit is stored at 2-8°C, and the expiration date is indicated on the label. Only properly stored reagents are guaranteed. If the components of the kit need to be used again, make sure that they have not been contaminated since the last use.

Store the unopened kit at 2-8°C.

Please use it within the validity period.

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Open

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Try

Agent 1 x lotion

1× detection buffer

Stop solution

Standard diluent

Chromogenic substrate TMB

Detection antibody

Horseradish peroxidase labeled

Streptavidin

Signal enhancer concentrate

Signal enhancer diluent at 2-8℃,

It can be stored for about 1 month.

The standard product can be stored for about 1 month at -20°C.

Discard after use.

Please put the unused slats back into the aluminum foil bag and seal the seal. It can be stored for about 1 month at 2-8°C.

4. Matters needing attention

1) All chemical reagents should be considered as potentially hazardous.

2) It is recommended that only staff with good laboratory training can operate this kit. Please wear suitable protective equipment during operation, such as white coat, latex gloves, safety glasses, etc.

3) Please avoid reagent contact with skin and eyes. In case of accidental contact, please wash immediately with plenty of water.

4) The stop solution in the kit is an acidic solution. When using the stop solution, please wear protective clothing and facilities to protect your eyes, hands and face.

5) This kit is used for scientific research and cannot be used for diagnosis and treatment.

6) Please do not use other batch numbers or reagents from other sources to replace the reagents in this kit.

7) Please do not use expired reagents.

8) Please avoid strong light during the storage or incubation of the kit.

9) Please do not eat or drink in the area where the kit is operated or the sample is processed.

10) Do not allow reagents or samples to contact the skin and

mucous membranes.

11) Please wear latex or disposable gloves when handling the kit or handling samples.

12) Avoid contact with oxidizing reagents and metals from the chromogenic substrate.

13) Avoid the generation of aerosols.

14) In order to avoid microbial contamination and cross-contamination between reagents and samples, please use disposable tips.

15) Use clean containers to prepare reagents.

16) Exposure to an acidic environment will inhibit binding.

17) The preparation of reagents must use distilled water or deionized water.

18) The chromogenic substrate must be equilibrated to room temperature before use.

19) The samples may contain infectious pathogens. The preferred method for handling samples and possible contaminated materials is 121.5°C for at least 1 hour.

20) Disposal of liquid waste. For liquid waste without acid, add 1.0% sodium hypochlorite and soak for 30 minutes. Please neutralize the acid-containing liquid waste before adding sodium hypochlorite.

21) Sometimes protein precipitation can be observed in the standard dilution solution, this precipitation does not affect the use and can be ignored. Alternatively, the precipitate can be removed by centrifugation at 6,000 × g for 5 minutes.

5. Technical points

1) When re-dissolving or mixing protein, always avoid air bubbles.

2) To avoid cross-contamination, please replace the pipette tip when adding standard samples, sample samples, and different reagents. Different reagents use different loading tanks.

3) When using an automatic plate washer, after adding the washing solution, set a 30-second soaking program, or turn the microplate 180 degrees in different washing steps, which can improve the accuracy of the analysis.

4) To ensure the accuracy of the results, seal the plate with the sealing film during incubation.

5) The chromogenic substrate should be colorless before adding. Keep the chromogenic substrate always in a light-proof state.

6) The order of addition of the stop solution should be the same as the order of addition of the chromogenic substrate.

7) After adding the stop solution, the color of the substrate should change from blue to yellow. If the substrate is green, it means that the stop solution and the color-developing substrate are not sufficiently mixed.

8) It is recommended that all test samples and standard products have multiple holes in the test.

9) In any case, avoid touching the inner surface of the microplate.

Three, detection steps

1. Sample collection and storage

Cell culture supernatant

Centrifuge at 300 × g for 10 minutes to remove the sediment, and then detect it immediately, or in aliquots, and store below -20°C.

Serum sample

Collect serum in centrifuge tube. After 30 minutes of agglutination, the blood sample was centrifuged at 1,000 × g for 10 minutes. Test immediately after drawing the serum sample, or aliquot and store it below -20°C.

Plasma sample

EDTA, sodium citrate or heparin anticoagulation to collect

plasma samples. Collect samples by centrifugation at 1,000 × g for 30 minutes. Immediately detect, or sub-package, and store below -20°C.

This kit may be suitable for other biological samples. Cell culture supernatant, serum and plasma have been validated.

Note: Before testing, the visible precipitation in the sample must be removed. Do not use samples with severe hemolysis or hyperlipidemia. The samples should be aliquoted and stored at -20°C to avoid loss of human G-CSF activity. If tested within 24 hours. The samples can be stored at 2-8°C.

Avoid repeated freezing and thawing of samples. Before testing, the frozen sample should be slowly returned to room temperature and mixed gently.

2. Reagent preparation

Please return all reagents and samples to room temperature before testing.

If the concentrated reagents crystallize, warm the bath at 37°C until all the crystals are dissolved.

1×Lotion

Draw 20×concentrated lotion from 50 ml to 1 L measuring cylinder, add distilled water to 1,000 ml, and mix gently to

avoid foam. Transfer to a clean bottle. Stored at 2-25℃, 1× lotion can be stored stably for 30 days.

1× detection buffer

Pipette 10× concentrated detection buffer from 5 ml to 100 ml graduated cylinder, add distilled water to 50 ml, and mix gently to avoid foam. Stored at 2-8℃, 1× detection buffer can be stored stably for 30 days.

Detection antibody

Mix well before dilution. Dilute the concentrated detection antibody 1:100 with 1× detection buffer according to the number of standards and samples to be tested.

Note: Please use the diluted detection antibody within 30 minutes.

Horseradish peroxidase labeled streptavidin

Mix well before dilution. Dilute the concentrated horseradish peroxidase-labeled streptavidin at a ratio of 1:100 with 1× detection buffer according to the number of standards and samples to be tested.

Note: Please use the diluted horseradish peroxidase-labeled streptavidin within 30 minutes.

Signal enhancer concentrate

Mix well before dilution. Dilute the concentrated signal

enhancer 1:100 with the signal enhancer diluent according to the number of standards and samples to be tested.

Note: Please use the diluted signal enhancer within 30 minutes.

Sample dilution

If the sample needs to be diluted, please dilute the serum/plasma sample with the 1× detection buffer provided in the kit, and dilute the cell culture supernatant with cell culture medium

Human G-CSF standard

Centrifuge briefly before opening the lid, re-dissolve the human G-CSF standard with distilled water, and the re-dissolved volume is marked on the label of the human G-CSF standard. Vortex gently to ensure thorough mixing. The concentration of the standard after re-dissolution is 2,000 pg/ml. After re-dissolving, let it stand for 10-30 minutes. Mix well before dilution.

Please use polypropylene tubing for standard dilution.

Preparation of the standard curve of serum/plasma samples:

Take 230 μl of concentrated human G-CSF standard and

add 230 µl of standard dilution as the highest concentration of the standard curve (1,000 pg/ml). Add 230 µl of standard diluent to each test tube. Use high-concentration standards to make 1:1 serial dilutions. Every time you pipette, make sure to mix well. Use the standard dilution as the zero concentration of the standard curve.

Preparation of the standard curve of cell culture supernatant samples:

Take 230 µl of concentrated human IL-33 standard and add 230 µl of cell culture medium as the highest concentration of the standard curve (400 pg/ml). Add 230 µl of cell culture medium to each test tube. Use high-concentration standards to make 1:1 serial dilutions. Make sure to mix well each time you pipette. Take the cell culture medium as the zero concentration of the standard curve.

3. Detection steps

Please equilibrate all reagents and samples to room temperature before testing.

1) Prepare all required reagents and working concentration standards.

2) Remove the unnecessary slats, put them back in the aluminum foil bag with desiccant, and re-seal the seal.

3) Soak the ELISA plate: Add 300 μl 1× lotion and let it stand and soak for 30 seconds. In order to obtain the desired experimental results, soaking is necessary. After discarding the lotion, pat the microplate dry on absorbent paper. After washing the plate, please use the microplate immediately and do not let the microplate dry.

4) Add standard product: add 100 μl standard product diluted 2 times to the standard product well. Add 100 μl of standard diluent (serum/plasma sample) or medium (cell culture supernatant sample) to the blank well.

5) Add sample: serum/plasma: add 50 μl 1× detection buffer and 50 μl sample to the sample hole. Cell culture supernatant:

add 100 μl of cell culture supernatant to the sample well. Ensure that steps 4 and 5 continue to add samples without interruption. The sample addition process is completed within 15 minutes.

6) Incubation: Use sealing film to seal the plate. Shake at 300 rpm and incubate at room temperature for 1.5 hours.

7) Washing: Discard the liquid, add 300 μl washing solution to each well to wash the plate, and wash 6 times. Each time you wash the plates, pat them dry on absorbent paper. In order to obtain the ideal experimental performance, the residual liquid must be completely removed.

8) Add detection antibody: add 100 μl diluted detection antibody (1:100 dilution) to each well. Use sealing film to seal the plate. Shake at 300 rpm and incubate at room temperature for 30 minutes.

9) Washing: Repeat step 7.

10) Incubation with enzyme: add 100 μl of diluted horseradish peroxidase-labeled streptavidin to each well (1:100 dilution). Seal the plate with a new sealing film. Shake at 300 rpm and incubate at room temperature for 30 minutes.

11) Washing: Repeat step 7.

12) Incubation with signal enhancer: add 100 μl of diluted

signal enhancer (1:100 dilution) to each well. Seal the plate with a new sealing film. Shake at 300 rpm and incubate precisely for 15 minutes at room temperature.

13) Washing: Repeat step 7.

14) Incubate with enzyme again: add 100 μl of diluted horseradish peroxidase-labeled streptavidin to each well (1:100 dilution). Seal the plate with a new sealing film. Shake at 300 rpm and incubate precisely for 15 minutes at room temperature.

15) Washing: Repeat step 7.

16) Add substrate for color development: add 100 μl of color substrate TMB to each well, protect from light, and incubate at room temperature for 5-30 minutes.

17) Add stop solution: add 100 μl stop solution to each well. The color changed from blue to yellow. If the color is green or the color change is obviously uneven, tap the board frame lightly to mix well.

18) Detection reading: within 30 minutes, use a microplate reader for dual-wavelength detection, and determine the OD value at 450 nm maximum absorption wavelength and 570 nm or 630 nm reference wavelength. The OD value after calibration is the measured value of 450 nm minus the

measured value of 570 nm or 630 nm. Using only 450 nm measurement will result in a high OD value and a decrease in accuracy.

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