RES107-EN.01

resDect™ Human G-CSF ELISA Kit
(Enzyme-Linked Immunosorbent Assay)

Catalog Number：RES-A107

Pack Size: 96 tests

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedure

US and Canada: **Tel**: +1 800-810-0816

Asia and Pacific: **Tel**: +86 400-682-2521

Web: http://www.acrobiosystems.com

E-mail: order@acrobiosystems.com

Content

INTENDED USE

The kit was developed for the detection and quantitative of G-CSF in human serum, plasma and cell culture supernatants. It is intended for research use only (RUO).

BACKGROUND

Granulocyte colony-stimulating factor (G-CSF or GCSF) is a glycoprotein, growth factor and cytokine produced by several different tissues to stimulate the bone marrow to produce granulocytes and stem cells. G-CSF can affect the hematopoietic system and neuronal cells as a neurotrophic factor. The action of G-CSF in the central nervous system is to induce neurogenesis, to increase the neuroplasticity and to counteract apoptosis. G-CSF stimulates the production of white blood cells.

To support the development of CAR-T drugs, ACROBiosystems developed Human G-CSF ELISA Kit with rigorous methodological validation, which is used evaluation the quality of CAR-T products in drug development and CMC quality control stages.

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of human G-CSF by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-G-CSF Antibody. Firstly, add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti-G-CSF Antibody to the plate and form Antibody-antigen-biotinylated antibody complex, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor solution color from blue to yellow. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm. The OD Value reflects the amount of human G-CSF bound.

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. The kit is suitable for cell supernatant, serum and plasma samples.
3. Do not use reagents past their expiration date.
4. Do not mix or substitute reagents with those from other kits or other lot number kits.
5. If the sample test value is higher than the concentration of the highest standard sample, dilute the sample with an appropriate calibrator diluent, and then retest it. If the cell culture supernatant sample requires serial dilution, except for the last step which uses the diluent for dilution, the cell culture medium can be used for the dilutions in other intermediate steps.
6. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage.
7. This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed

MATERIALS PROVIDED

Table1. Materials provided

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RES107-C01	Pre-coated Anti-G-CSF Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RES107-C02	Human G-CSF Standard	15 μg	Powder	2-8°C	-70°C
RES107-C03	Biotin-Anti-G-CSF Antibody	20 μg	Powder	2-8°C	-70°C
RES107-C04	Streptavidin-HRP	50 μL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES107-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RES107-C06	2×Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RES107-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES107-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

Note: *It is recommended that Streptavidin-HRP be centrifuged briefly before use to deposit liquid from the tube wall or cap to the bottom of the tube.*

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

STORAGE

1. Unopened kit should be stored at 2°C -8°C upon receiving.
2. The opened kit should be stored per Table 1. The shelf life is 30 days from the date of opening.

Note:

a. Do not use reagents past their expiration date.

b. Find the expiration date on the outside packaging.

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

1. Single or multi-channel micropipettes and pipette tips: need to meet 10 µL, 300 µL, 1000 µL injection requirements;
2. 37ºC Incubator;
3. Single or dual wavelength microplate reader with 450 nm and 630 nm filter;
4. Tubes: 1.5mL,10mL;
5. Timer;
6. Reagent bottle;
7. Deionized or distilled water.

US and Canada: **Tel**: +1 800-810-0816
Asia and Pacific: **Tel**: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

QUICK GUILD

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in an 37℃ incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.
2. According to Table 2, prepare the provided lyophilized product into a storage solution with ultrapure water, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vertexing. The reconstituted storage solution should be stored at -70°C. It is recommended that the number of freezing and thawing should not exceed 1 time, the size of the aliquot should not be less than 5 μg.

Note: *Considering inevitable minor quantitation variations between protein batches, it is also reasonable to generate the standard curve with specific lot of proteins used for current production for even better accuracy.*

Table 2. Preparation method

ID	Components	Size (96 T)	Storage solution concentration.	Reconstituted water Vol.
RES107-C02	Human G-CSF Standard	15 μg	150 μg/mL	100 μL
RES107-C03	Biotin-Anti-G-CSF Antibody	20 μg	200 μg/mL	100 μL

RECOMMENDED SAMPLE PREPARATION

1. **Working Solution Preparation**

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

1.3 Preparation of Biotin-Anti-G-CSF Antibody working fluid:

Dilute Biotin-Anti-G-CSF Antibody to 0.2 μg/mL with 1×Dilution Buffer. Please prepare it for one-time use only.

1.4 Preparation of Streptavidin-HRP working fluid:

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

Dilute Streptavidin-HRP at 1:2000 with 1×Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

1.5 Sample preparation

a. If the sample to be tested is the cell supernatant, dilute test sample at 1:2 with 1×Dilution Buffer. The volume ratio of sample to diluent is 1:1.

b. If the sample to be tested is the serum/plasma, dilute test sample at 1:10 with 1×Dilution Buffer. The volume ratio of sample to diluent is 1:9.

2. Preparation of Standard curve

The concentration of the reconstituted human G-CSF Calibrator (RES107-C02) is 150 μg/mL，prepare (Std.-0) by diluting 10 μL the reconstituted human G-CSF Calibrator into 990 μL Sample Dilution Buffer, mix gently well. Then prepare Std.- 1' by diluting 10 μL Std.-0 into 990 μL Sample Dilution Buffer. At last, prepare the highest concentration of standard curve, Std.-1 (300 pg/mL), by diluting 12 μL Std.- 1' into 588 μL Sample Dilution Buffer. Prepare 1:1 serial dilution for the standard curve as follows: Pipette 300 μL of Sample Dilution Buffer into each tube. Make sure to mix well every time. Sample Dilution Buffer serves as blank.

Tubes/ Solution Code	Human G-CSF Standard stock solution	Std.-0	Std.-1'	Std.-1	Std.-2	Std.-3	Std.-4	Std.-5	Std.-6
Operating	10μL	10 μL	12 μL	300 μL	300 μL	300 μL	300 μL	300 μL	
Solution Con.	150μg/mL	1500 ng/mL	15 ng/mL	300 pg/mL	150 pg/mL	75 pg/mL	37.5 pg/mL	18.75 pg/mL	9.375 pg/mL
Dilution Buffer Vol.		990 μL	990 μL	588 μL	300 μL	300 μL	300 μL	300 μL	300 μL

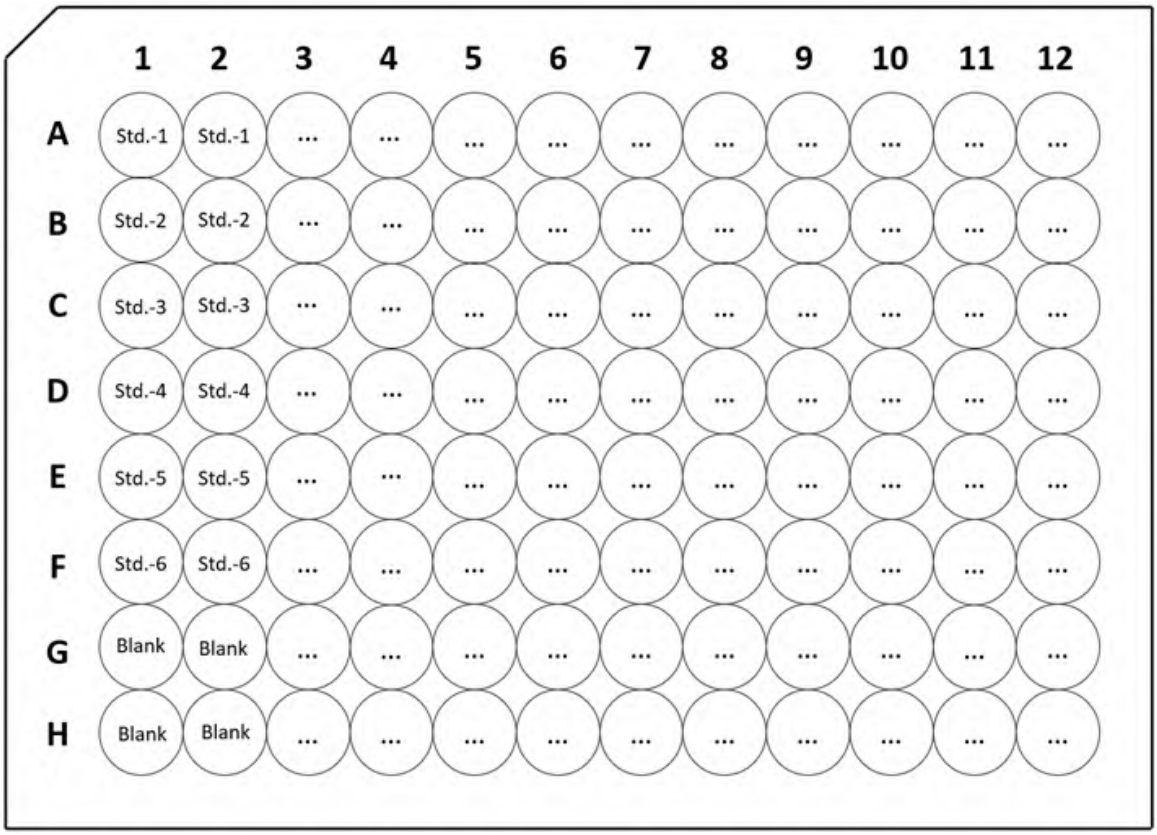
US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

3. Add Samples

Add 100 µL Calibrator and samples to each well. For blank Control wells, please add 100 µL Dilution Buffer.

***Note**:*

1. *It is recommended to set duplicate for samples and standard curves to be tested.*
2. *Blank is a Blank Dilution Buffer hole.*



4. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

5. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 10 s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

6. Add Biotin-Anti-G-CSF Antibody

For all wells, add 100 µL Biotin-Anti-G-CSF Antibody (dilute to 0.2 µg/mL) working solution. Please prepare it for one-time use only.

7. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1.0 hour.

8. Washing

Repeat step 5.

9. Add Streptavidin-HRP

For all wells, add 100 µL Streptavidin-HRP (dilute at 1:2000) working solution. Please prepare it for one-time use only, avoid light.

10. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 30 min.

11. Washing

Repeat step 5.

12. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

13. Termination

Add 50 µL Stop Solution to each well and tap the plate gently to allow thorough mixing

Note: *The color in the wells should change from blue to yellow.*

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

14. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 10 minutes.

***Note**: To reduce the background noise, subtract the value read at OD_{450nm} with the value read at $OD_{630\ nm}$.*

CALCULATION OF RESULTS

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (OD).

2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.

3. Normal range of Standard curve: $R^2 \geq 0.9900$.

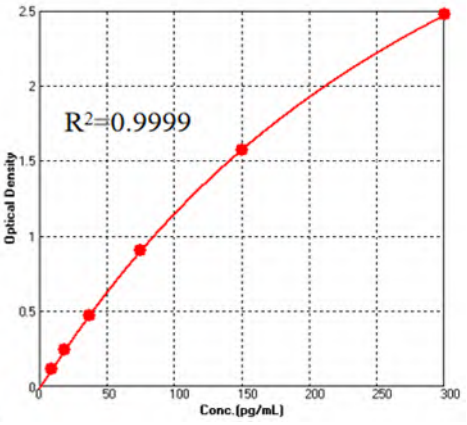
4. Detection range: 9.375 pg/mL-300 pg/mL. If the OD value of the sample to be tested is higher than 800 pg/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 9.375 pg/mL, the sample should be reported.

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

TYPICAL DATA

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipment. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Standard (pg/mL)	O.D.-1	O.D.-2	Average	Corrected
300	2.503	2.512	2.508	2.472
150	1.553	1.670	1.612	1.576
75	0.980	0.909	0.945	0.909
37.5	0.518	0.500	0.509	0.474
18.75	0.289	0.272	0.281	0.245
9.375	0.152	0.150	0.151	0.116
0	0.036	0.035	0.035	/



SENSITIVITY

The minimum detectable concentration of G-CSF is 4.267 pg/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

PRECISION

1. Intra-assay Precision

Three samples of known concentration were tested ten times on one plate to assess intra-assay precision.

2. Inter-assay Precision

Three samples of known concentration were tested in three separate assays to assess inter-assay precision.

Precision	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	3	3	3
Mean (pg/mL)	226.785	58.344	25.361	224.779	57.242	23.940
SD	8.355	2.443	1.326	4.487	1.235	1.389
CV (%)	3.7	4.2	5.2	2.0	2.2	5.8

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

***Note**: The example data is for reference only.*

RECOVERY

Three samples with different concentrations were tested to calculate the recovery rate.

Sample(n=5)	Average Recovery %	Range %
High	93.2	87.9-108.8
Middle	94.8	84.2-102.2
Low	96.9	83.0-104.1

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of G-CSF were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture medium (DMEM)	Cell culture medium (1640)	Serum	EDTA Plasma	Heparin Plasma	Citrate Plasma
1:2	Average Recovery %	92.5	88.2	95.1	94.4	103.8	104.1
	Range (%)	87.3-94.6	87.3-90.3	90.0-97.7	88.3-103.0	96.0-112.4	99.6-107.9
1:4	Average Recovery %	89.8	86.6	106.6	91.4	95.4	100.6
	Range (%)	88.2-91.1	83.4-90.6	103.3-111.3	86.4-99.2	91.7-100.2	91.1-106.2
1:8	Average Recovery %	91.8	94.0	113.4	98.5	110.7	108.4
	Range (%)	86.4-95.1	87.7-97.8	107.5-116.2	92.6-106.6	105.2-114.8	100.0-115.2
1:16	Average Recovery %	90.7	98.6	115.7	105.1	110.9	105.0
	Range (%)	87.8-96.8	87.0-109.9	114.6-116.7	99.5-112.6	103.7-115.4	90.1-114.2

***Note**: The example data is for reference only.*

SPECIFICITY

This assay recognizes natural and recombinant human G-CSF. No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines.

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

Human	Mouse
IL-3 M-CSF GM-CSF IL-3 R alpha	IL-3 GM-CSF

SAMPLE VALUES

Human peripheral blood mononuclear cells (5×10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum,100 U/mL penicillin and 100 µg/mL streptomycin sulfate and stimulated with 0.1 µg/mL LPS. Aliquots of the culture supernatant were removed on days 1 and 5 and assayed for levels of human G-CSF.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	1340.844	340.316
Stimulated	3570.154	5837.727

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human G-CSF (09/136). Reference Reagent is calibrated by NIBSC/WHO in April 2013.

NIBSC/WHO (09/136) approximate value (U/mL) = 1.107× Human G-CSF value (pg/Ml)

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of theassay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com