Speedy™ Human/Cynomolgus Monkey G-CSF One-Step ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: SE50251

Size: 96T

Sensitivity: 11.0 pg/mL

Range: 31.25-2000 pg/mL

Usage: For the quantitative detection of human/cynomolgus monkey G-CSF concentrations in serum, plasma and cell culture supernatants.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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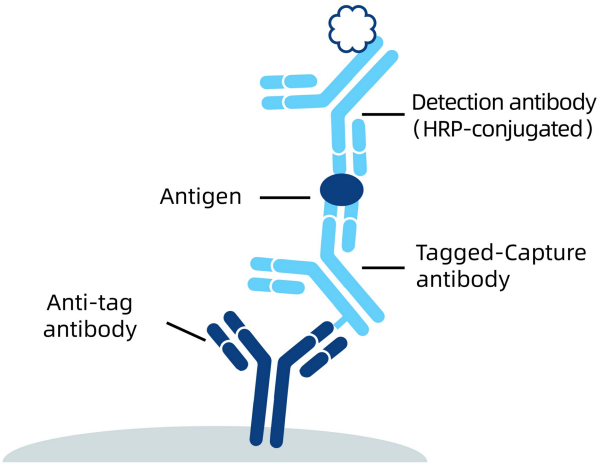
1. Background

Granulocyte colony-stimulating factor (G-CSF), also referred to as CSF3, is a protective cytokine with anti-inflammatory effects. G-CSF is important in promoting survival of the granulocytic lineage cells and proliferation and migration of neutrophils as well as trophoblast cells. G-CSF acts by binding to its receptor G-CSFR (also called CSF3R), which after binding with G-CSF activates the canonical Janus kinase (Jak)/signal transducer, activator of transcription (STAT)and Ras/Raf/MAP kinase pathways. G-CSF potently stimulates the proliferation and release of peripheral blood progenitor cells into the bloodstream and is therefore used to treat neutropenia after chemotherapy. Furthermore, G-CSF levels are elevated upon intensive exercise leading to increased neutrophil counts, which are predominantly due to delayed neutrophil apoptosis.

2. Principle



An anti-tag antibody is pre-coated onto the bottom of wells. After adding antigen or samples, Tagged-Capture antibody and HRP-conjugated detection antibody, a sandwich complex is formed in the solution. TMB acts as a HRP substrate, and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns the solution yellow. The color intensity is proportional to the quantity of bound protein, which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended. Proteintech data analysis website, https: //www.ptgcn.com/products/elisa-kits/.

3.8 Microplate thermostatic shaker.

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4. Kit Components and Storage

Microplate - 96 well microplate precoated an anti-tag antibody (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 4000 pg/bottle; lyophilized	2 bottles	
Capture antibody (100×) - 60 uL/vial*	1 vial	
Detection antibody, HRP-Conjugated (100×) - 60 uL/vial*	1 vial	
Sample Diluent PT 3 - 30 mL/bottle; For human serum and plasma.	1 bottle	
Sample Diluent PT 4B1 - 30 mL/bottle; For cell culture supernatants.	1 bottle	
Detection Diluent - 15 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

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7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Antibody Cocktail (1X): Dilute 100X capture antibody and 100X HRP-conjugated detection antibody using Detection Diluent prior to assay. Suggested 1:100 dilution: 50 µL 100X capture antibody + 50 µL 100X Detection Antibody, HRP-conjugated + 4,900 µL Detection Diluent. Mix gently but thoroughly.

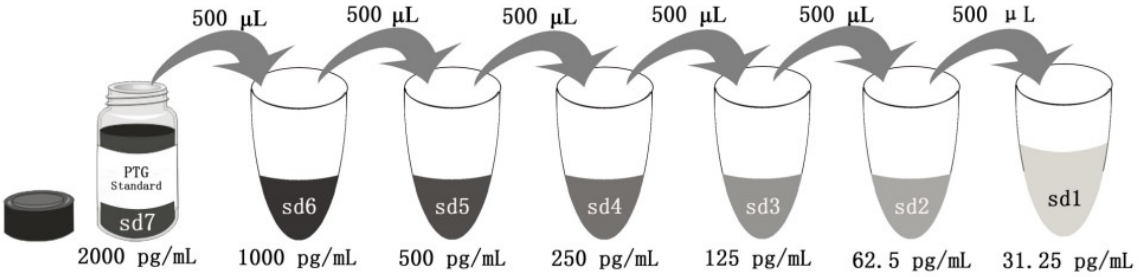
7.3 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent. The minimum required dilution factor was 1:2; consequently, no more than 25 µL of undiluted sample was added per well.

Samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:2 or 1:4 is recommended for human serum, plasma and human cell culture supernatant; 1:16 or 1:32 is recommended for cynomolgus monkey cell culture supernatant.

7.4 Standard Serial Dilution:

For human serum and plasma, add 2mL Sample Diluent PT 3 in protein standard. For cell culture supernatants, add 2mL Sample Diluent PT 4B1 in protein standard.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 3 or PT 4B1	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated antibody can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Preset the layout of the microplate, including control group, standard group and sample group, take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Add 50 µL standard or sample to appropriate wells. To avoid high background always add samples or standards to the well before the addition of antibody cocktail.

8.3 Add 50 µL 1× Antibody Cocktail solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 1 hour (incubate at 37°C for 2 hours is recommended if thermostatic shaker is not available) .

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1× Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL TMB substrate solution to each well, protected from light. Incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 15 to 20 minutes. (Substrate Solution should remain colorless until added to the plate.)

8.6 Add 100 µL Stop Solution to each well in the same order as addition of the TMB substrate. Note: Avoid skin and eye contact with the Stop solution.

8.7 Read results immediately on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.8 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, four-parameter logistic curve- fit (4-PL) analysis is recommend. If the samples have been diluted, the fitting result must be multiplied by the dilution factor used.

Procedure summary

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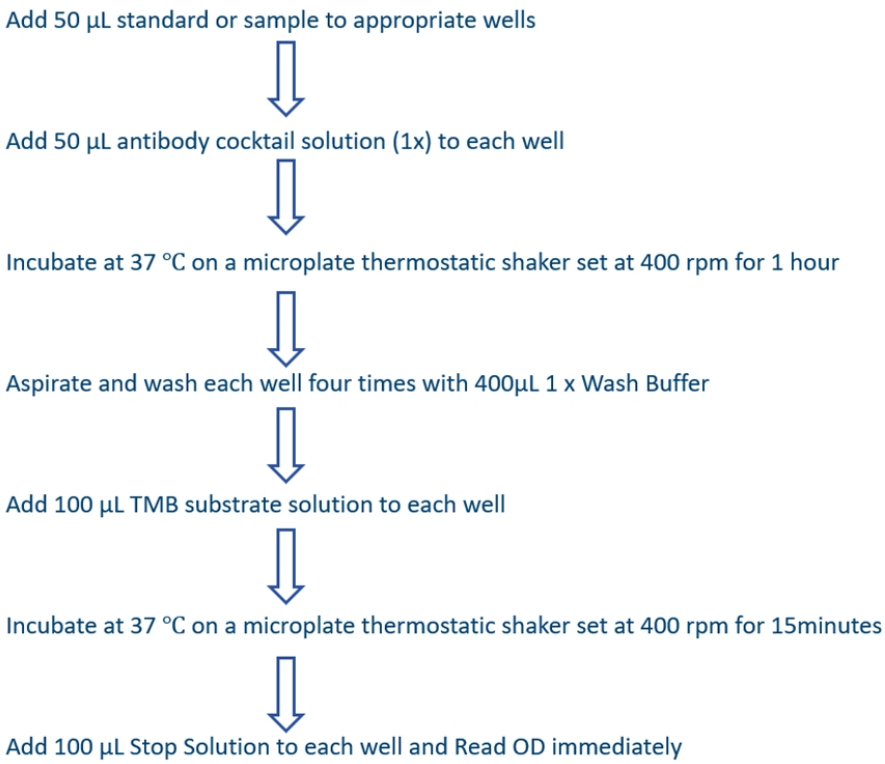

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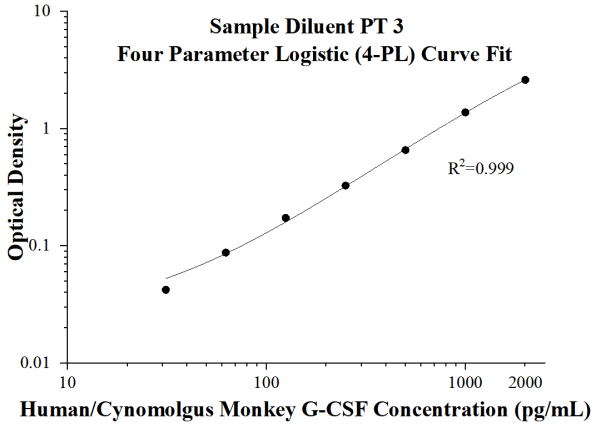
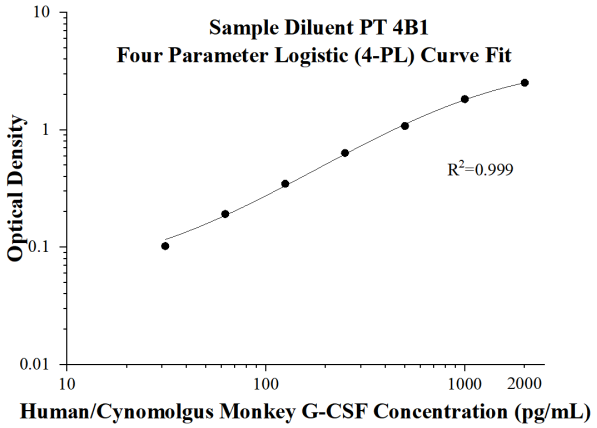
9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.0284 0.0289	0.0287	-
31.25	0.0714 0.0704	0.0709	0.0423
62.5	0.1174 0.1153	0.1164	0.0877
125	0.2066 0.1968	0.2017	0.1731
250	0.3767 0.335	0.3559	0.3272
500	0.7211 0.6505	0.6858	0.6572
1000	1.4159 1.3961	1.4060	1.3774
2000	2.664 2.6149	2.6395	2.6108



(pg/mL)	O.D	Average	Corrected
0	0.0378 0.0357	0.0368	-
31.25	0.142 0.1358	0.1389	0.1022
62.5	0.2302 0.2271	0.2287	0.1919
125	0.3861 0.3817	0.3839	0.3472
250	0.6742 0.6705	0.6724	0.6356
500	1.1022 1.1316	1.1169	1.0802
1000	1.9527 1.7825	1.8676	1.8309
2000	2.5502 2.5658	2.5580	2.5213

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9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	8	975.6	21.1	2.2
2	8	224.3	4.9	2.2
3	8	112.8	3.3	2.9

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	16	968.2	18.1	1.9
2	16	222.2	4.5	2.0
3	16	111.5	3.1	2.8

9.3 Recovery

The recovery of human/cynomolgus monkey G-CSF spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human plasma	1:2	77	71-81
Human cell culture supernatant	1:4	100	93-110
	1:8	101	98-106
Cynomolgus monkey cell culture supernatant	1:32	100	95-102
	1:64	100	95-103

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9.4 Sample values

Human plasma - human plasma samples were evaluated for the presence of G-CSF in this assay.

Sample Type	Mean (pg/mL)	% Detectable	Range (pg/mL)
Human plasma (n=16)	62.5	50	ND-235.1

ND*=Non-detectable

Cell culture supernatant:

Human peripheral blood mononuclear cells (PBMC) (1 x 10^6 cells/mL) were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, 5μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μg/mL PHA for 1 day and 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of G-CSF.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	ND	ND
Stimulated	834.2	4,155.8

ND*=Non-detectable

Cynomolgus monkey peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented 10% fetal bovine serum, 5 μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μg/mL PHA for 3 days and 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of G-CSF.

Condition	Day 3 (**ng/mL**)	Day 5 (**ng/mL**)
Unstimulated	0.3	0.2
Stimulated	14.4	13.4

9.5 Sensitivity

The minimum detectable dose of human/cynomolgus monkey G-CSF is 11.0 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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9.6 Linearity

To assess the linearity of the assay, human plasma samples were spiked with high concentrations of human/cynomolgus monkey G-CSF and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Cell culture supernatants samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(The cynomolgus monkey cell culture supernatant was initially diluted 1:8.)

		Human plasma (Sample Diluent PT 3)	Human cell culture supernatant (Sample Diluent PT 4B1)	Cynomolgus monkey cell culture supernatant (Sample Diluent PT 4B1)
1:2	Average% of Expected	80	100	100
	Range (%)	77-83	-	-
1:4	Average% of Expected	98	95	93
	Range (%)	94-101	94-96	89-97
1:8	Average% of Expected	95	90	90
	Range (%)	94-96	90-91	87-93
1:16	Average% of Expected	82	88	89
	Range (%)	77-86	86-91	85-92

9.7 Calibration

The NIBSC/WHO International Standard for Human/Cynomolgus monkey G-CSF (09/136), which was intended as a potency standard, was evaluated in this kit. The dose response curve of the International Standard (09/136) parallels the Proteintech standard curve. To convert sample values obtained with the Human/Cynomolgus monkey G-CSF ELISA kit to approximate NIBSC 09/136 units, use the equation below.

NIBSC (09/136) approximate value (IU/mL)=0.05530x Proteintech Human/Cynomolgus monkey G-CSF value (pg/mL)

9.8 Specificity

This assay recognizes natural and recombinant human/cynomolgus monkey G-CSF.

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:	Recombinant mouse:
GM-CSF	G-CSF
IL-3	IL-3
IFN-a/b R1	
IL-3 Rα	

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10. References

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