

Code No. 27131

Human G-CSF Assay Kit - IBL

INTRODUCTION

G-CSF is the cytokine that stimulates differentiation and propagation of neutrophil precursor cells. It is produced by fibroblast and endothelial cells and it is reported to stimulate the propagation in some tumor. Human G-CSF Assay Kit (L) - IBL can be measured Human G-CSF.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetra Methyl Benzidine (TMB) is used as coloring agent (Chromogen). The strength of coloring is in proportion to the quantities of Human G-CSF.

MEASUREMENT RANGE

7.81 ~ 500 pg/mL (1st incubation for 1 hour at 37°C)
 1.95 ~ 250 pg/mL (1st incubation for overnight at 4°C) : In-house data

INTENDED USE

- The IBL's Human G-CSF Assay Kit is a complete Kit for the quantitative determination of Human G-CSF supernatant of cell culture media.
- Both recombinant and native forms of human G-CSF can be detected with the kit.
- Our in-house experiment suggested that the assay in low diluted serum or EDTA - plasma samples may cause the lower values due to the interference of substances in blood. (Please see "Table. 1.Titer Assay, PERFORMANCE CHARACTERISTICS" on page 2.)

KIT COMPONENT

1	Precoated plate	: Anti- Human G-CSF Mouse IgG MoAb Affinity Purify	96Well x 1
2	Labeled antibody Conc.	: (30X) HRP conjugated Anti-Human G-CSF Rabbit IgG Fab' Affinity Purify	0.4mL x 1
3	Standard	: Recombinant Human G-CSF	1.0mL x 2
4	EIA buffer *		30mL x 1
5	Solution for Labeled antibody *		12mL x 1
6	Chromogen	: TMB solution	15mL x 1
7	Stop solution *		12mL x 1
8	Wash buffer Conc. *		50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

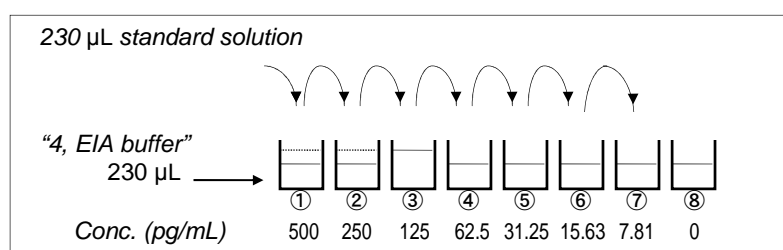
- Plate reader (450nm)
- Graduated cylinder and beaker
- Incubator (37°C ± 1°C)
- Graph paper (log/log)
- Tube for dilution of Standard
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"
- Micropipette and tip
- Distilled water
- Paper towel
- Washing bottle for precoated plate

2. Preparation

- 1) Preparation of wash buffer
 "8, Wash buffer Conc." is a concentrated (40X) buffer. The temperature of "8, Wash buffer Conc." shall be adjusted to room temperature and then, mix it gently and completely before use. Dilute 50mL of "8, Wash buffer Conc." with 1,950mL of distilled water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.
- 2) Preparation of Labeled antibody
 "2, Labeled antibody Conc." is a concentrated (30X). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.
 Example)
 In case you use one slit (8 well), the required quantity of Labeled antibody is 800 µL. (Dilute 30 µL of "2, Labeled antibody Conc." with 870 µL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 µL in each well.)
This operation should be done just before the application of Labeled antibody.
 The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.
- 3) Preparation of Standard
 Put just 1.0 mL of distilled water into the vial of "3, Standard" and mix it gently and completely. This solution is 1,000 pg/mL Human G-CSF standard.
- 4) Dilution of Standard
 Prepare 8 tubes for dilution of "3 Standard". Put 230 µL each of "4, EIA buffer" into the tube.
 Specify the following concentration of each tube.

Tube-1	500 pg/mL
Tube-2	250 pg/mL
Tube-3	125 pg/mL
Tube-4	62.5 pg/mL
Tube-5	31.25 pg/mL
Tube-6	15.63 pg/mL
Tube-7	7.81 pg/mL
Tube-8	0 pg/mL (Test Sample Blank)

Put 230 µL of Standard solution into tube-1 and mix it gently. Then, put 230 µL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 500 pg/mL and 7.81 pg/mL. Tube-8 is the test sample blank as 0pg/mL. See following picture.



In-house procedure (1st incubation for overnight at 4°C)

If high sensitivity is need, below is followed.

- ① Preparation of Standard
 Put just 2.0 mL of distilled water into the vial of "3, Standard" and mix it gently and completely. This solution is 500 pg/mL Human G-CSF standard.
- ② Dilution of Standard
 Prepare 9 tubes for dilution of "3 Standard". Put 230 µL each of "4, EIA buffer" into the tube.
 Specify the following concentration of each tube.

Tube-1	250 pg/mL
Tube-2	125 pg/mL
Tube-3	62.5 pg/mL
Tube-4	31.25 pg/mL
Tube-5	15.63 pg/mL
Tube-6	7.81 pg/mL
Tube-7	3.91 pg/mL
Tube-8	1.95 pg/mL
Tube-9	0 pg/mL (Test Sample Blank)

 Put 230 µL of Standard solution into tube-1 and mix it gently. Then, put 230 µL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 8 points of diluted standard between 250 pg/mL and 1.95 pg/mL. Tube-9 is the test sample blank as 0 pg/mL.
- 5) Dilution of test sample
 Test sample may be diluted with "4, EIA buffer" if the need arises. If the concentration of Human G-CSF in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Confirm no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

Reagents	Test Sample	Standard	Test Sample Blank	Reagent Blank
	Test sample 100 µL	Diluted standard (Tube 1~7) 100 µL	EIA buffer (Tube-8) 100 µL	EIA buffer 100 µL
Incubation for 1 hour at 37°C with plate lid (In-house procedure : Incubation for overnight at 4°C with plate lid)				
4 times (wash buffer more than 350 µL) *				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30minutes at 37°C with plate lid				
5 times (wash buffer more than 350 µL) *				
Chromogen	100 µL	100 µL	100 µL	100 µL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 µL	100 µL	100 µL	100 µL
Read the plate at 450nm against a Reagent Blank within 30 minutes after application of Stop solution.				

- 1) Determine wells for reagent blank. Put 100 µL each of "4, EIA buffer" into the wells.
- 2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 µL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1~7) into the appropriate wells. (In-house procedure : put 100 µL each of test sample blank (tube-9), test sample and dilutions of standard (tube-1~8) into the appropriate wells.)
- 3) Incubate the precoated plate for 1 hour at 37°C (In-house procedure : overnight at 4°C) after covering it with plate lid.
- 4) Wash the plate with the prepared wash buffer and remove all liquid. *
- 5) Pipette 100 µL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
- 6) Incubate the precoated plate for 30 minutes at 37°C after covering it with plate lid.
- 7) Wash the plate with the prepared wash buffer and remove all liquid. *
- 8) "6, Chromogen" should be taken the required quantity into a disposable test tube. Then, pipette 100 µL from the test tube into the wells. Please avoid to return the rest of test tube into "6, Chromogen" bottle due to avoid to cause of contamination.
- 9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by the addition of "6, Chromogen".

- 10) Pipette 100 µL of "7, Stop solution" into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by the addition of "7, Stop solution".
- 11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the liquid. Then, run the plate reader and conduct measurement at 450nm. The measurement shall be done within 30minutes after the addition of "7, Stop solution".

SPECIAL ATTENTION

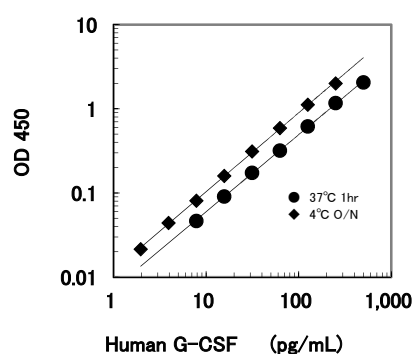
1. Test samples should be measured soon after the collection. In case of the storage of test samples, they should be stored under frozen conditions and do not repeat freeze/thaw cycles. Thaw the test samples at low temperature and mix them completely before measurement.
2. Test samples should be diluted with "4, EIA buffer", if the need arises.
3. The measurement of test samples and standard in duplicate is recommended.
4. Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
5. Use only wash buffer contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
6. Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
7. "6, Chromogen" should be stored in the dark due to its sensitivity against light. "6, Chromogen" should be avoided contact with metals.
8. Measurement should be done within 30 minutes after addition of "7, Stop solution".

CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

Conc. (pg/mL)	Absorbance (450nm)	
	1 st incubation	
	37°C 1hr	4°C O/N (In-house)
500	2.068	—
250	1.184	2.022
125	0.633	1.139
62.5	0.334	0.612
31.25	0.189	0.334
15.63	0.106	0.181
7.81	0.062	0.103
3.91	—	0.066
1.95	—	0.043
0 (Test Sample Blank)	0.015	0.022



The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS (As assay of 1st incubation for 1 hour at 37°C)

1. Titer Assay (Samples with standard added are used.)

Specimen	Titer (X)	Measurement Value(pg/mL)	Theoretical Value(pg/mL)	%
10% FCS added RPMI-1640	2	112.86	125	90.3
	4	56.49	62.5	90.4
	8	28.31	31.25	90.6
Human Serum	8	215.33	500	43.1
	16	163.16	250	65.3
	32	100.62	125	80.5
	64	54.19	62.5	86.7
Human Plasma (EDTA)	8	263.45	500	52.7
	16	173.30	250	69.3
	32	99.18	125	79.3
	64	55.77	62.5	89.2

2. Added Recovery Assay

Specimen	Theoretical Value (pg/mL)	Measurement Value (pg/mL)	%
10% FCS added RPMI-1640 (x2)	500.07	452.07	90.4
	250.07	233.25	93.3
	125.07	116.15	92.9
Human Serum (x32)	125.95	97.53	77.4
	63.45	48.89	77.1
	32.20	24.51	76.1
Human Plasma (EDTA) (x32)	125.07	91.62	73.3
	62.57	48.33	77.2
	31.32	23.79	76.0

3. Intra - Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
229.96	17.32	7.5	24
49.51	2.89	5.8	24
10.45	0.76	7.3	24

4. Inter- Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
227.83	15.76	6.9	32
48.98	2.94	6.0	32
10.35	0.75	7.2	32

5. Specificity

Compound	Cross Reactivity
Human G-CSF	100.0 %
Human GM-CSF	≤0.1 %

6. Sensitivity

1.23 pg/mL

The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2~8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. "3, Standard" is lyophilized products. Be careful to open this vial.
3. "7, Stop solution" is a strong acid substance. Therefore, be careful not to contact your skin and clothes with "7, Stop solution" and pay attention to the disposal of "7, Stop solution".
4. Dispose used materials after rinsing them with large quantity of water.
5. The precipitation may grow in "2, Labeled antibody Conc.", however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from different lot or different kit.
8. Do not use the reagents expired.
9. This kit is for research purpose only. Do not use for clinical diagnosis.
10. The data obtained at 4°C overnight on primary incubation is in-house data.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8 °C
The expiry date is specified on outer box.

Version 2.

September 2016 *

Made in Japan.