KRIBIOLISA™ PEG-GCSF (Pegylated Filgrastim) ELISA

REF : KBBA10

Ver1.1

Enzyme Immunoassay for the Quantitative Determination of PEG-GCSF in serum, plasma and cell culture supernatant

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF **KBBA10**  **96 tests**

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Introduction:

Pegfilgrastim is a recombinant PEGylated human granulocyte colony-stimulating factor (G-CSF) analog filgrastim (trade names Neulasta® or G-Lasta®) that stimulates the production of white blood cells (neutrophils). It is employed as an alternative to filgrastim (G-CSF) for chemotherapy-induced neutropenia in patients due to its longer half-life.

Intended Use:

The KRIBIOLISA™ PEG-GCSF (PEG-Filgrastim) ELISA is specifically designed for the accurate quantitation of human PEG-GCSF from cell culture supernatant, serum, plasma and other bodily fluids. It is ready-to-use, accurate, and sensitive.

Materials Provided:

Part	Description	Qty
Microtiter Coated Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Peg-Filgrastim monoclonal Antibody.	1 x 96 wells
Recombinant human PEG-GCSF Standard	Lyophilized Human PEG-GCSF Standard Concentration - 1 ug/ml.	2 vials
Anti- PEG-GCSF: HRP Conjugate	Anti-PEG-GCSF: HRP Conjugate prepared in buffer with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	12 ml
(1X) Sample Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	50 ml
(1X) Standard Diluent	Buffered protein base with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane with 1:100 dilution human serum	10 ml
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersol < 0.01%. May turn yellow over time.	25 ml
TMB Substrate	Stabilized Chromogen	12 ml
Stop Solution	2N Sulfuric Acid	12 ml
Instruction Manual		1 no

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.

Storage Information:

1. Store main kit components at 2-8°C.
2. Store recombinant **Standard at 2-8°C**. Upon reconstituting, aliquot recombinant protein into polypropylene vials and store at -20°C as per assay requirements. Do not freeze thaw for more than two times.
3. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Sample Preparation and Storage:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided.

Samples should be diluted 1:100 (v/v) for optimal recovery, (for example 1 ul sample + 99 ul (1X) Sample Diluent) prior to assay. In cases where matrix interferences is under or over observed, the samples may be diluted with Sample Diluent accordingly.

The samples may be kept at 2 - 8°C for up to three days. For long-term storage please store at -20°C.

Note: *Grossly hemolyzed samples are not suitable for use in this assay*

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20℃ or lower temperature. Avoid repeated freeze-thaw cycles. If the use of original supernate sample or low dilution (<5 fold) are necessary due to the expected low concentration of antigen supernates need be adjust to neutral pH condition before assay.

Note: The sample should be diluted to within the working range of the assay in 1X Sample Diluent. The exact dilution must be determined based on the concentration of specific target in individual samples.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room Temperature before use.
3. To make **Wash Buffer (1X)**; dilute **25 ml** of **20X Wash Buffer** in **475 ml of DI water**.
4. **Standards Preparation**: Reconstitute the concentrated Standard lyophilized vial with 1 ml of Standard Diluent to obtain a concentration of 1ug/ml. Keep the vial for 15 mins with gentle agitation before making further dilutions. Dilute 160 ul of original **Standard (1 ug/ml)** with 840 ul of Standard Diluent to generate a **160 ng/ml Standard Solution**. Prepare further **Standards** by serially diluting the Standard Solution as per the below table. Use the Standard Diluent as the Zero Standard (Standard No.0).

Standard Concentration	Standard No	Dilution Particulars
1 ug/ml	Main Stock, lyophilized	Reconstitute with 1ml of Standard Diluent
160 ng/ml	Standard No.6	160 ul of Main Stock + 840 ul of Standard Diluent
80 ng/ml	Standard No.5	500 ul Standard No.6 + 500 ul Standard Diluent
40 ng/ml	Standard No.4	500 ul Standard No.5 + 500 ul Standard Diluent
20 ng/ml	Standard No.3	500 ul Standard No.4 + 500 ul Standard Diluent
10 ng/ml	Standard No.2	500 ul Standard No.3 + 500 ul Standard Diluent
5 ng/ml	Standard No.1	500 ul Standard No.2 + 500 ul Standard Diluent
0 ng/ml	Standard No.0	250 ul Standard Diluent

Assay Procedure:

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
2. Add **100 ul** of **Standards or samples** to the respective wells.
3. Cover the plate with a sealer. Incubate for 1 hour at 37° C.
4. Aspirate and wash plate 4 times with diluted **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
5. Add **100 ul** of **Anti- PEG-GCSF: HRP Conjugate** solution to each well.
6. Cover the plate with a sealer. Incubate at 37°C for 1 hour.
7. Wash plate 4 times with (1X) **Wash Buffer** as in step 4
8. Add **100 ul** of **TMB Substrate** solution and incubate at 37°C 30 minutes. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.
9. Add **100 ul** of **Stop Solution** to each well. Positive wells should turn from blue to yellow.
10. Read absorbance at 450 nm within 30 minutes of stopping reaction.

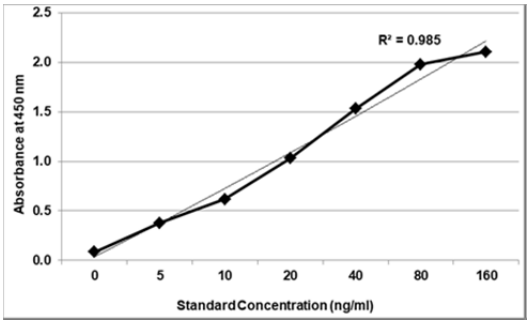
Calculation of Results:

Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Using semi log graph paper or computer programs, plot the optical densities of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit straight line through the standard points. To determine the unknown PEG-GCSF concentrations, find the unknowns mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the PEG-GCSF concentration. If samples were diluted, multiply by the appropriate dilution factor. Computer based curve-fitting software may be preferred.

Typical Data

Standard Concentration (ng/ml)	Abs A	Abs B	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.089	0.080	0.085	--	--
5	0.389	0.366	0.377	5.6	111.3
10	0.621	0.616	0.618	10.0	99.7
20	1.027	1.036	1.032	19.4	96.8
40	1.516	1.547	1.532	39.2	98.0
80	1.953	2.008	1.980	92.1	115.1
160	2.103	2.109	2.106	138.3	86.5

Typical Graph



Abs = absorbance @450nm

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics of the Kit:

This kit has been validated as per EMA/FDA guidelines in line with ICH Code for Harmonization of Biological Assays.

Sensitivity:
Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD.
10 replicates of '0' standards were evaluated and the LOD was found to be 5 ng/ml

Precision:
Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low (5ng/ml), medium (20ng/ml) and high (160ng/ml) concentrations. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Pool	Intra Assay %CV	Inter Assay %CV
Low	<10%	<10%
Medium	<5%	<5%
High	<5%	<5%

Safety Precautions:

- **This kit is for research use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves
  - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

Components (2-8ºC) —30mins, RT Before Use→ 

Thaw at Room Temperature (18-24ºC) → USE NOW

2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components → **AVOID** → **COOL (2-8ºC)** → **THAW (22±4ºC)** → **AVOID** → **COOL (2-8ºC)** → **THAW (22±4ºC)** → **AVOID** → NO CHANGE IN RESULTS

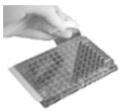
3.  Pipette **100 ul Standards** / **Samples** into the respective wells

4. Cover plate  and incubate for 

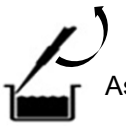
at 37°C.

5.  Aspirate and wash wells 4 times with **Wash Buffer (1X)**

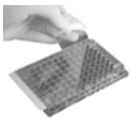
6.  Pipette **100 ul** of **Anti- PEG-GCSF: HRP Conjugate** into the respective wells.

7. Cover plate  and incubate for 

at 37°C.

8.  Aspirate and wash wells 4 times with **Wash Buffer (1X)**

9.  Pipette **100 ul TMB Substrate** into each well.

10. Cover plate  and incubate for 

at 37°C

11.  Pipette **100 ul Stop Solution** into each well.

12. Read absorbance at 450nm with a  microplate reader within 

of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	ng/ml PEG-GCSF equivalent
1A 2A	0 ng/ml 0 ng/ml			
1B 2B	5 ng/ml 5 ng/ml			
1C 2C	10 ng/ml 10 ng/ml			
1D 2D	20 ng/ml 20 ng/ml			
1E 2E	40 ng/ml 40 ng/ml			
1F 2F	80 ng/ml 80 ng/ml			
1G 2G	160 ng/ml 160 ng/ml			
1H 2H	Sample			
3A 4A	Sample			
3B 4B	Sample			

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SYMBOLS KEY

MTP	Microtiter Coated Plate (12x8 wells)
STD	Human PEG-GCSF Standard, lyophilized
HRP CONJ	HRP Conjugate Detection Antibody
1X STD DIL	(1X) Standard Diluent
1X SAMP DIL	(1X) Sample Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature