KRIBIOLISA™ GCSF (FILGRASTIM) ELISA

Cat. No: KBBA14

Ver1.1

ELISA Set for Accurate Quantitation from Cell Culture Supernatant, Serum, Plasma, or Other Bodily Fluids

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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RE **KBBA14** Σ **96 tests**

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Introduction:

Filgrastim, sold under the brand name Neupogen among others, is a medication used to treat low neutrophil count. Low neutrophil counts may occur with HIV/AIDS, following chemotherapy or radiation poisoning, or be of an unknown cause. It may also be used to increase white blood cells for gathering during leukapheresis. It is given either by injection into a vein or under the skin.

Common side effects include fever, cough, chest pain, joint pain, vomiting, and hair loss. Severe side effects include splenic rupture and allergic reactions. It is unclear if use in pregnancy is safe for the baby. Filgrastim is a recombinant-DNA form of the naturally occurring granulocyte colony-stimulating factor (G- CSF). It works by stimulating the body to increase neutrophil production.

Intended Use:

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

Materials Provided:

1. Microtiter Coated Plate (8x12 wells) – 1 no
2. Recombinant human GCSF Standard (60ng/ml, lyophilized) - 1 vial
3. Biotinylated GCSF Detection Antibody - 1 vial
4. Streptavidin: Horseradish Peroxidase - 1 vial
5. (20X) Wash Buffer - 25 ml
6. (1X) Assay Diluent - 50 ml
7. (1X) Detection Diluent – 12 ml
8. TMB Substrate - 12 ml
9. Stop Solution - 12 ml
10. Instruction Manual

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.

Specimen Collection and Handling:

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

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -20°C. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x *g*. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -20°C. Avoid repeated freeze/thaw cycles.

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

The (1X) Assay Diluent recommended may be suitable for most cell culture supernate, serum, and plasma samples. The (1X) Assay Diluent optimization for samples with complex matrices such as serum and plasma, may improve their performance in this assay. We recommend using 1:10 to 1:100 as the serum / plasma dilution ratio with the (1X) Assay 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. **Standards Preparation:** Reconstitute the lyophilized vial with 100 ul of Distilled water to generate a 60 ng/ml standard solution. Keep the standard for 15 mins with gentle agitation before making further dilutions. Perform serial dilutions by using main stock solution as per the below table. Thus the Human GCSF Standard concentration are 8000 pg/ml, 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml and 125 pg/ml. Assay Diluent (1X) serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
60 ng/ml	Main Stock, lyophilized	Reconstitute with 100 ul Distilled water
8000 pg/ml	Standard No. 7	66.7 ul of Main Stock + 433.3 ul of Assay Diluent (1X)
4000 pg/ml	Standard No.6	250 ul Standard No.7 + 250 ul Assay Diluent (1X)
2000 pg/ml	Standard No.5	250 ul Standard No.6 + 250 ul Assay Diluent (1X)
1000 pg/ml	Standard No.4	250 ul Standard No.5 + 250 ul Assay Diluent (1X)
500 pg/ml	Standard No.3	250 ul Standard No.4 + 250 ul Assay Diluent (1X)
250 pg/ml	Standard No.2	250 ul Standard No.3 + 250 ul Assay Diluent (1X)
125 pg/ml	Standard No.1	250 ul Standard No.2 + 250 ul Assay Diluent (1X)
0 pg/ml	Standard No.0	250 ul Assay Diluent (1X)

3. Add **100 ul** of **Standards or samples** to the respective wells.

4. Cover the plate with a sealer. Incubate for 2 hours at room temperature.

5. 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.

6. Add **100 ul** of diluted **Biotinylated GCSF Detection Antibody** solution to each well.
7. Cover the plate with a sealer. Incubate at room temperature for 2 hours.
8. Wash plate 4 times with (1X) **Wash Buffer** as in step 5.
9. Add **100 ul** of diluted **Streptavidin-HRP** solution to each well.
10. Cover the plate with a sealer. Incubate at room temperature for 30 minutes.
11. Wash plate 4 times with 1X **Wash Buffer** as in step 5.
12. Add **100 ul** of **TMB Substrate** solution and incubate at RT in the dark for 30 minutes. Positive wells should turn bluish in color. It is not necessary to seal the plate during this step.
13. Add **100 ul** of **Stop Solution** to each well. Positive wells should turn from blue to yellow.
14. Read absorbance at 450 nm within 30 minutes of stopping reaction.

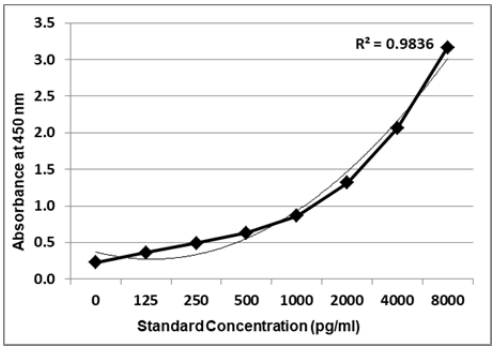
Calculation of Results:

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 cytokine 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 cytokine concentration. If samples were diluted, multiply by the appropriate dilution factor. Computer based curve-fitting software may be preferred.

Typical Data

Standard Concentration (pg/ml)	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.229	--	--
125	0.361	115.5	92.4
250	0.489	289.6	115.8
500	0.630	517.0	103.4
1000	0.865	957.6	95.8
2000	1.314	1967.7	98.4
4000	2.060	4051.8	101.3
8000	3.162	7984.5	99.8

Typical Graph



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.

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.

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

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THANK YOU FOR USING KRISHGEN PRODUCT!

SYMBOLS KEY

MTP	Anti-Human GCSF Microtiter Plate (12x8 wells)
STD	Human GCSF Standard, lyophilized
BIO CONJ	Biotin Conjugated Detection Antibody
STRP HRP	Streptavidin Horseradish Peroxidase
BIO CONJ DIL	(1X) Biotin Conjugation Diluent
1X ASY DIL	(1X) Assay 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