MATRIKS BIOTEK
innovation for health & wellness

"trace & catch"

Instructions For Use

Antibody to Filgrastim

SHIKARI®
Q-AFA

Enzyme immunoassay for the quantitative determination of antibodies to Filgrastim in serum and plasma with confirmation reagent-ligand binding Nab assay

REF **TR-AFAv1** 12 x 8 2-8°C

Revision # 1.3 August 2017

CE
IVD

Matriks Biotek® Laboratories
www.matriksbiotek.com

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	SHIKARI Q-AFA
	Filgrastim antibodies quantitative analyses
Required Volume (µl)	10
Total Time (min)	200
Sample	Serum, plasma
Sample Number	96
Detection Limit (ng/mL)	31
Spike Recovery (%)	Between 85-115
Shelf Life	6 months

Intended Use

The *Matriks Biotek®* Antibody to Filgrastim (ATF) Enzyme-Linked- Immuno-Sorbent-Assay (ELISA) Kit is intended for the quantitative determination of antibodies to filgrastim in serum and plasma. It is for professional use only. The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations.

Summary and Explanation

Filgrastim is a recombinant, non-pegylated human granulocyte colony stimulating factor (G-CSF) analogue manufactured by recombinant DNA technology using a strain of E. coli. Chemically, it consists of 175 amino acid residues. The protein has an amino acid sequence that is identical to the natural sequence predicted from human DNA sequence analysis, except for the addition of an N-terminal methionine necessary for expression in E coli.

Filgrastim binds to the G-CSF receptor and stimulates the production of neutrophils in the bone marrow. As a G-CSF analog, it controls proliferation of committed progenitor cells and influences their maturation into mature neutrophils. Filgrastim also stimulates the release of neutrophils from bone marrow storage pools and reduces their maturation time. Filgrastim acts to increase the phagocytic activity of mature neutrophils. In patients receiving cytotoxic chemotherapy, Filgrastim can accelerate neutrophil recovery, leading to a reduction in duration of the neutropenic phase.

Filgrastim is used in patients with acute myeloid leukemia receiving induction or consolidation chemotherapy. It is also used in cancer patients receiving bone marrow transplant. In general, filgrastim increases neutrophil counts in order to decrease the risk of infection or duration of neutropenia in the aforementioned patient populations. Infection and neutropenia are adverse events associated with chemotherapy. Furthermore, filgrastim is also indicated for patients with severe chronic neutropenia. It mobilizes hematopoietic progenitor cells into the peripheral blood for collection by leukapheresis to allow for a more rapid engraftment.

Used in the treatment of chemotherapy-induced neutropenia by enhancing the production of neutrophils. Filgrastim acts on hematopoietic cells by binding to specific cell surface receptors thereby stimulating proliferation, differentiation, commitment, and end cell functional activation. When tbo-filgrastim is administered to cancer patients, it took 3-5 days to reach maximum absolute neutrophil count (ANC). Levels of neutrophils returned to baseline by 21 days following completion of chemotherapy. In the healthy volunteer trials, doubling the tbo-filgrastim subcutaneous dose from 5 to 10 mcg/kg resulted in a 16-19% increase in the ANCmax and a 33-36% increase in the area under the effect curve for ANC.

Test Principle

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the double antigen assay principle. Diluted standards and samples (serum or plasma) are incubated in the microtitre plate coated with filgrastim. After incubation, the wells are washed. A biotin conjugated filgrastim is added and binds to ATF captured by the reactant on the surface of the wells. Following incubation, wells are washed and then streptavidine–horse radish peroxidase (HRP) is added and binds to biotin conjugated tracer. After incubation, the wells are washed and the bound enzymatic activity is detected by addition of chromogen-substrate. The colour developed is proportional to the amount of filgrastim in the sample or standard. Results of samples can be determined directly using the standard curve. Detection depends on double antigen ELISA plus with special reagent to detect monomeric IgG4 antibodies covering all class of anti-drug antibodies.

Warnings and Precautions

1. For professional use only.

2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood. For further information (clinical background, test performance, automation protocols, alternati e applications, literature, etc.) please refer to the local distributor.

3. In case of severe damage of the kit package please contact Matriks Biotek® or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.

4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.

5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.

6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details.

7. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guidelines or regulations

8. Avoid contact with Stop solution. It may cause skin irritations and burns

9. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with large volume of water to avoid azide build-up.

10. All reagents of this test kit containing human serum or plasma have been tested and were found negative for HIV I/II, HBsAg and HCV by FDA approved procedures. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

Storage and Stability

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.

The strips of microtiter plate is stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8°C.

Specimen Collection and Storage

Serum, Plasma (EDTA, Heparin)*

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	-20°C	Keep away from heat or direct sun light
Stability:	7 d	6 mon	Avoid repeated freeze-thaw cycles

*. Filgrastim infusion camouflages/masks the presence of antibody to Filgrastim in serum/plasma samples. Therefore, blood sampling time is critical for detection of Filgrastim. Matriks Biotek® Laboratories propose to obtain blood sample just before the infusion of Filgrastim or at least 2 weeks after the infusion of Filgrastim.

Materials Supplied

1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with filgrastim.
8 x 0.3 mL	STND A-F HIGH CNTRL LOW CNTRL	**Filgrastim Standards A-F (10X), High Level Control, Low Level Control** 500; 250; 125; 62; 31; 0 ng/mL Ready to use. Used for construction of the standard curve. Contains human serum, antibody to filgrastim, and <0.1% NaN_3.
1 x 30 mL	ASSAY BUF	**Assay Buffer,** Concentrate (5x) Blue colored. Ready to use. Contains proteins and <0.1% NaN_3.
1 x 150 µL	BIOTIN CONJ	**Biotin-Labeled Filgrastim,** Concentrate (100x) Dilute only required amount of biotinylated conjugate not before than 30 minutes. Contains biotin labeled Filgrastim and <0.1% NaN_3.
1 x 12 mL	STREP-HRP	**Streptavidine-Peroxidase** Red colored. Ready to use. Contains streptavidine conjugated to horse radish peroxidase (HRP) and stabilizers.
1 x 12 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains TMB
1 x 12 mL	TMB STOP	**TMB Stop Solution** Ready to use. 1N HCl.
1 x 50 mL	WASHBUF CONC	**Wash Buffer**, Concentrate (20x) Contains Buffer with Tween 20.
2 x 1	ADH FILM	**Adhesive Film** For covering of Microti er Plate during incubation
1 x 210 µL	CONF RGNT	**Confirmation Reagent** (For 20 Samples) Ready to use. Contains optimized concentration of the filgrastim and <0,1% NaN_3.

Materials Required But Not Supplied

1. Micropipettes (< 3% CV) and tips to deliver 5-1000 µL.
2. Calibrated measures.
3. Tubes (1 mL) for sample dilution.

4. Wash bottle, automated or semi-automated microtiter plate washing system.

5. Microtiter plate reader capable of reading absorbance at 450/650 nm.

6. Bidistilled or deionised water, paper towels, pipette tips and timer.

Procedure Notes

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.

2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.

3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.

4. Use a pipetting scheme to verify an appropriate plate layout.

5. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.

6. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipeĀtte or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.

7. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

Preparation of Component

Dilute/ disolve	Component	with	Diluent	Relatio	Remarks	Storage	Stability
10 mL	Wash Buffer*	Up to 200 mL	bidist. Water	1:20	Warm up at 37°C to dissolve crystals. Mix vigorously.	2-8 °C	2 w

*****Prepare Wash Buffer before starting assay procedure.

2.Dilution of Standards and Samples (serum/plasma)

Sample	To be diluted	With	Remarks
Standards	1:10	Assay Buffer	For dilution at 1:10; 20 µl Standard + 180 µl Assay Buffer
Serum/Plasma	1:1000	Assay Buffer	First; for dilution at 1:10; 10 µl Standard + 90 µl Assay Buffer Second; for dilution at 1:100; 5 µl Standard + 495 µl Assay Buffer

In case of samples still being higher than the "Highest Standard (Standard A)"should be further diluted with assay buffer and retested.

PREPRATION OF BIOTIN-LABELED CONJUGATE

Dilute/ disolve	Component	with	Diluent	Relation	Remarks	Storage	Stability
10 µL	Biotin-labeled Conjugate*	Up to 1000 µL	Assay Buffer	1:100	Warm up at 37°C to dissolve crystals. Mix vigorously.	2-8 °C	30 min

*****Dilute only required amount of biotinylated conjugate not before than 30 minutes.

PREPRATION OF CONFIRMATION TEST MIXTURE (FOR 1 SAMPLE)

Sample	**Dilute**	**In**	**Remarks**
Diluted Positive Serum/Plasma AND Confirmation Reagent	10 µl's from each	100 µl Assay Buffer	Incubate 60 minutes and use 100 µl from this 120 µl solution for the assay

Test Procedure

1	**Dilute each of the standards and samples (serum/plasma) using Assay Buffer** as described in "Dilution of Standards and Samples (serum/plasma)" section.
2	Pipette 100 µL of each Diluted Standards, High Level Control, Low Level Control or diluted Serum Samples into the respective wells of microtiter plate. Wells A1: Standard A B1: Standard B C1: Standard C D1: Standard D E1: Standard E F1: Standard F G1: High Level Control H1: Low Level Control A2 and on: Sample (Serum/Plasma)
3	Cover the plate with adhesive film. **Incubate 60 min** at room temperature (18-25°C).
4	Remove adhesive film. Dis ard incubation solution. Wash plate **3 times** each with **300 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
5	Pipette **100 µL** of ready-to use **Biotin-Labeled Filgrastim** into each well.Dilute only required amount of biotinylated conjugate not before than 30 minutes.
6	Cover the plate with adhesive film. **Incubate 60 min** at room temperature (18- 25°C).
7	Remove adhesive film. Discard incubation solution. Wash plate **3 times** each with **300 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
8	Pipette **100 µL** of ready-to use **Streptavidine-Peroxidase** into each well.
9	Cover the plate with a new adhesive film. **Incubate 60 min** at room temperature (18-25°C).
10	Remove adhesive film. Discard incubation solution. Wash plate **3 times** each with **300 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
11	Pipette **100 µL** of **TMB Substrate Solution** into each well.
12	**Incubate 20 min** (without adhesive film) at room temperature (18-25°C) in the dark.
13	Stop the substrate reaction by adding **100 µL** of **Stop Solution** into each well. Briefly mix contents by gently shaking the plate. Color changes from blue to yellow.
14	**Measure** optical density with a photometer at **450/650 nm** within **30 min** after pipetting of the Stop Solution.

Confirmation Test

	CONFIRMATION TEST FOR POSITIVE SAMPLES
	Incubate positive patient samples and optimized confirmation reagent for **60 minutes in a microtube**. After the incubation proceed the test procedure from step one given above, by pipetting 100 µl of this solution to respective well. Instructions are followed given in the **test procedure** in table.

QUALITY CONTROL

The test results are only valid only if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards must be found within the acceptable ranges as stated above. If the criteria are not met, the run is not valid and should be repeated. In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

Calculation & Interpretation of Results

QUANTITATIVE INTERPRETATION

1. Using the diluted standards (50, 25, 12.5, 6.2, 3.1 ng/mL) disregarding zero standard, construct a standard curve by plotting the OD450/650 nm for each of 5 standards on the vertical (Y-axis) axis versus the corresponding ATF concentration on the horizontal (X-axis) axis, thus creating a standard curve by 4 points obtained.

2. The concentration of the samples can be read directly from this standard curve. Using the absorbance value for each sample, determine the corresponding concentration of ATF from the standard curve. Find the absorbance value on the Y-axis and extend a horizontal line to the curve. At the point of intersection, extend a vertical line to the X-axis and read the ATF concentration for the unknown sample.

3. If computer data regation is going to be used, we recommend primarily "4 Parameter Logistic (4PL)" or secondly the "point-to-point calculation".

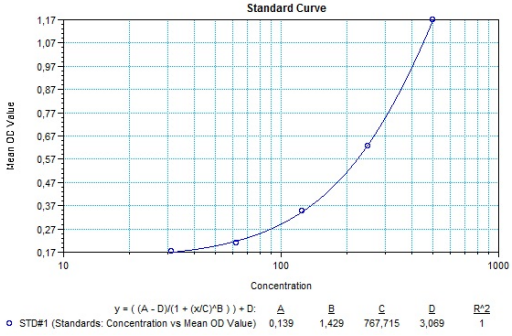
4. To obtain the exact values of the samples, the concentration determined from the standard-curve must be multiplied by the dilution factor (100x). Any sample reading greater than the highest standard should be further diluted appropriately with Assay Buffer and retested. Therefore, if the pre-diluted samples have been further diluted, the concentration determind from the standard curve must be multiplied by the further dilution factor.

E.g.; *If the pre-diluted sample further diluted in a ratio of 1:10 then results should be multiplied by 1000.*

5. Automated method: Computer programs can also generally give a good fit.

Typical Calibration Curve

(Example. Do not use for calculation!)



Standard	Concentration (ng/mL)	Mean $OD_{450/650}$
A	50	1,169
B	25	0,628
C	12,5	0,348
D	6,2	0,211
E	3,1	0,173
F	0	0,100

Assay Characteristics

1. Specificity: Filgrastim infusion camouflages/masks the presence of antibody to filgrastim (ATF) in serum/plasma samples. Therefore, blood sampling time is critical for detection of ATF. It is convenient to obtain blood sample just before the infusion or at least 2 weeks after the infusion of Filgrastim.

2. **Sensitivity:**The lowest detectable level that can be distinguished from the zero standard is 31 pg/mL.

3. **Precision of Kit:**
   Intra-assay CV: <15% for the ATF range of 31-500 ng/mL.
   Inter-assay CV: <15% for the ATF range of 31-500 ng/mL.

4. **Recovery:** Recovery rate was found to be between 85-115% when spiked using normal human serum samples with known concentrations.

Automation

Experiments have shown that the ***Matriks Biotek*® SHIKARI® Q-AFA ELISA** is also suitable to run on an automated ELISA processor.

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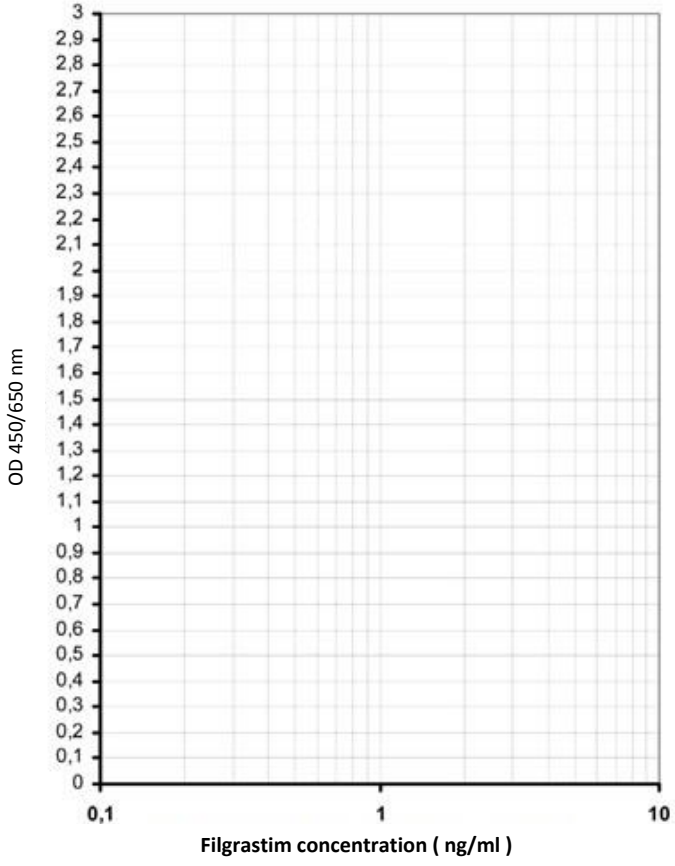
OD 450/650 nm
3
2,9
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1,1
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0,8
0,7
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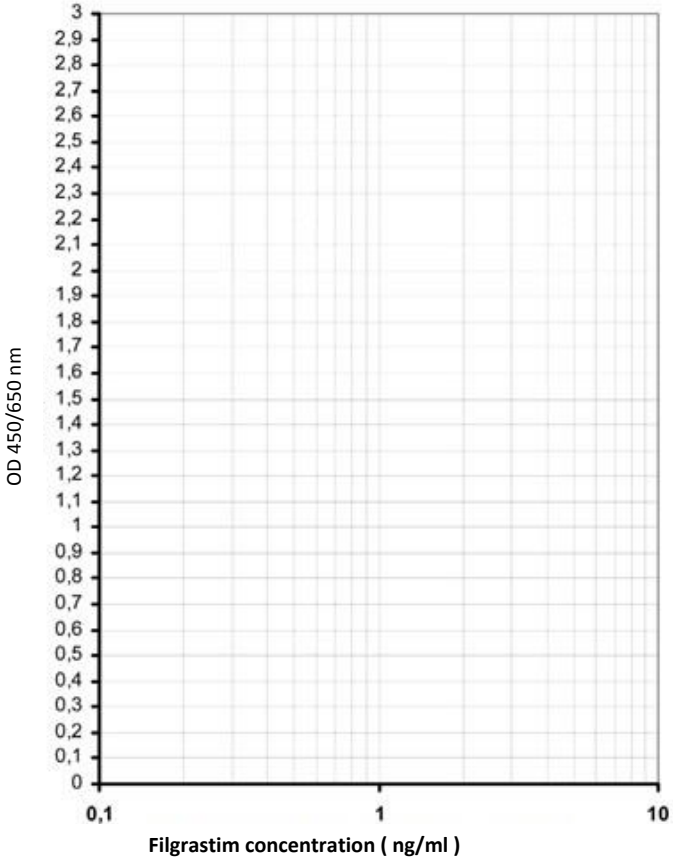
0,4
0,3
0,2
0,1
0
0,1
1
10
Filgrastim concentration (ng/ml)


OD 450/650 nm
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10
Filgrastim concentration (ng/ml)