BioSim™ anti-Filgrastim (Human) ELISA Kit

rev 02/21

(Catalog # E4399-100, 100 assays, Store at 4°C)

I. Introduction:

Filgrastim is a recombinant, non-pegylated human granulocyte colony-stimulating factor (G-CSF) analog. It binds to the G-CSF receptor and stimulates the production of neutrophils in the bone marrow. As a G-CSF analog, it controls the proliferation of committed progenitor cells and influences their maturation into mature neutrophils. Filgrastim is used in patients with acute myeloid leukemia receiving induction or consolidation chemotherapy. It is also used in cancer patients receiving bone marrow transplants. However, some patients develop unwanted immunogenicity, which leads to the production of anti-drug antibodies (ADAs) inactivating the therapeutic effects of the treatment and, in rare cases, inducing adverse effects. BioVision's BioSimTM anti-Filgrastim (Human) ELISA kit is designed to detect antibodies against Filgrastim with high specificity and sensitivity in biological matrices. The kit is based on the sandwich principle. Controls and samples are incubated in the microtiter plate coated with the drug Filgrastim. After incubation, the wells are washed, and the enzymatic activity is detected by the addition of chromogen-substrate. The enzyme-substrate reaction is terminated with an acidic stop solution. The color developed is proportional to the amount of antibodies specific for Filgrastim present in the samples and standards. The quantitative test results can be determined using the standard curve.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of antibody against Filgrastim in serum and plasma
Detection Range: 3.1 - 50 ng/ml
Sensitivity: 3.1 ng/ml
Cross Reactivity: Filgrastim infusion camouflages/masks the presence of antibody to Filgrastim (ATT) in serum/plasma samples. Therefore, blood sampling time is critical for detection of ATT. It is convenient to obtain blood sample just before the infusion of Filgrastim or at least 2 weeks after the infusion of Filgrastim.

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

Components	E4399-100	Part No.
Micro ELISA Plate	1 plate	E4399-100-1
Filgrastim Standards (S1 – S8)	0.3 ml X 8	E4399-100-2.x
Assay Buffer	2 x 50 ml	E4399-100-3
Peroxidase-Conjugate	12 ml	E4399-100-4
TMB substrate (Avoid light)	12 ml	E4399-100-5
Stop Solution	12 ml	E4399-100-6
Wash buffer (20X)	50 ml	E4399-100-7
Confirmation Reagent	1 ml	E4399-100-8
Plate sealers	2	E4399-100-9

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment.

VII. Reagent and Sample Preparation:

Note: Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer**: Dilute the 20X Wash Buffer to 1X solution in ddH_2O (10 ml of Wash Buffer stock to 190 ml of ddH_2O). Mix the 1X solution thoroughly by vortex manually. The working stock can be stable for 2 weeks after preparation at 4°C.
2. **Peroxidase-Conjugate**: Dissolve 10 µl of Peroxidase-Conjugate with 990 µl Assay Buffer (1:100 dilution) 30 minutes before the experiment. Warm up at 37°C to dissolve crystals. Mix vigorously if necessary.
3. **Standard Preparation:**
   Dilute Standards 10X with assay buffer (1: 10 dilution: 20 µL standard/control + 180 µL assay buffer)

Name	S1	S2	S3	S4	S5	S6	S7	S8
Conc. (ng/ml)	500	250	125	62.5	31.2	0	High Control	Low Control
Working Conc. (ng/ml)	50	25	12.5	6.25	3.12	0	-	-

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4. **Sample Dilution:**
   - **Serum/Plasma**: First dilute samples at 1:10 (10 µl Serum/Plasma + 90 µl ddH_2O) then 1:100 (5 µl diluted sample + 495 µl ddH_2O) to get the final samples with dilution factor 1:1000.
   - Diluted samples should further be diluted if the concentration of Filgrastim is higher than the measuring range.
   - The usual precautions for venipuncture should be observed. Samples are stable at 4°C for 7 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.
5. **Confirmation Test Mixture:** Mix 10 µl (positive) serum/plasma diluted sample (1:1000) with 10 µl confirmation reagent and 100 µl Assay Buffer for 60 minutes in a microtube prior to the test. Total volume: 120 µL. The purpose of performing a confirmation test is to confirm that the anti-drug antibodies in positive samples are true positives. In the case of true positives, the inhibition % of the reaction would be atleast 25% after incubation with the confirmation reagent.

VIII. Assay Protocol:

Note: Bring all reagents, microplate and samples to room temperature 15 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Add 100 µl of **diluted standards, diluted-samples**, and **confirmation test mixture** (if applicable) into appropriate wells. Cover wells and incubate for 60 minutes at room temperature (RT).
3. 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.
4. Add 100 µl of **Peroxidase-Conjugate** into each well. Cover wells with adhesive plate sealer and incubate at RT for 60 minutes.
5. Discard the solution and wash the wells as step 3.
6. Add 100 µl of **TMB substrate** solution and incubate the plate in dark at RT for 20 minutes
7. Add 100 µl of **Stop solution** to stop the reaction
8. Read the absorbance in micro plate reader set to 450 nm within 20 minutes. (reference wavelength to 650 nm)

IX. QUANTITATIVE CALCULATION:

Using the standards disregarding zero standard, construct a standard curve by plotting the OD 450/650 nm for each standard on the Y-axis versus the corresponding Filgrastim concentration on the X-axis. Construct a standard curve of difference data using software capable of generating four parameter logistic (4PL) or point-to-point calculation curve fit. To obtain the exact values of the samples, the concentration determined from the standard-curve should be multiplied by the dilution factor.

For the interpretation of true and false positives, use the following formula

$$\frac{\text{OD 450/650 (Sample)} - \text{OD 450/650 (Confirmation test mixture)}}{\text{OD 450/650 (Sample)}} \times 100 = \textbf{Inhibition \%}$$

If the **Inhibition %** is ≥ 25%, then the sample is a "true positive" for anti-drug antibody

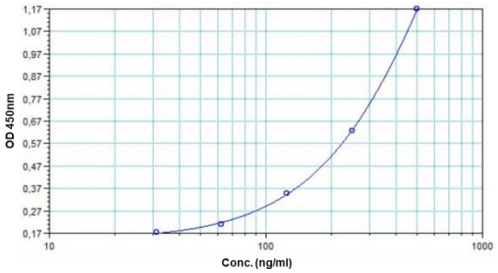

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

X. RELATED PRODUCTS:

- BioSim™ Rituximab (Human) ELISA Kit (Cat. No. E4385-100)
- BioSim™ Trastuzumab (Human) ELISA Kit (Cat. No. E4386-100)
- BioSim™ Infliximab (Human) ELISA Kit - I (Cat. No. E4387-100)
- BioSim™ Adalimumab (Human) ELISA Kit (Cat. No. E4388-100)
- BioSim™ Bevacizumab (Human) ELISA Kit (Cat. No. E4389-100)
- BioSim™ Infliximab (Human) ELISA Kit - II (Cat. No. E4390-100)
- BioSim™ Cetuximab (Human) ELISA Kit (Cat. No. E4391-100)
- BioSim™ Etanercept (Human) ELISA Kit (Cat. No. E4392-100)
- BioSim™ Golimumab (Human) ELISA Kit (Cat. No. E4393-100)
- BioSim™ Denosumab (Human) ELISA Kit (Cat. No. E4394-100)
- BioSim™ Omalizumab (Human) ELISA Kit (Cat. No. E4395-100)
- BioSim™ Nivolumab (Human) ELISA Kit (Cat. No. E4396-100)

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