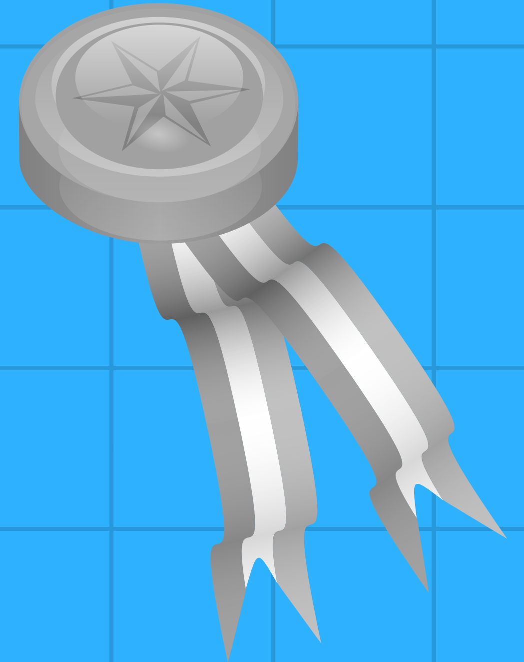

CATALOGUE SKU: LTFGM96

ANTI FILGRASTIM ELISA KIT

INSTRUCTION MANUAL

For quantitative determination
of specific antibodies to Filgrastim
in human serum and plasma samples

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Life Technologies® Platinum Series Anti-Filgrastim ELISA Kit

for quantitative determination of specific antibodies to filgrastim

in human serum and plasma samples.

INSTRUCTION MANUAL

FOR ELISA KIT No: LTFGM96

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INTENDED USE

This kit is used for quantitative determination of specific antibodies to filgrastim in serum and plasma samples. Developed and optimized using Fraven®. For *in vitro* use only.

MANUAL VERSION 1.01

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ASSAY SUMMARY

Chemotherapy-induced neutropenia (CIN) is a common and serious complication of myelosuppressive chemotherapy. It is associated with significant morbidity and mortality and can increase the cost of cancer therapy. In these cases, colony stimulating factor is necessary to restore important cells for immune function. Granulocyte colony-stimulating factors have been a pillar of treatment and prevention of CIN, and have been found to reduce the risk of neutropenia across various patient settings, decrease the incidence of febrile neutropenia, reduce the incidence of infection, reduce the requirement for treatment with antibiotics, and accelerate neutrophil recovery. Therapeutic drug monitoring (TDM) is the clinical practice of measuring specific drugs at designated intervals to maintain a constant concentration in a patient's bloodstream, thereby optimizing individual dosage regimens. The indications for drug monitoring include efficacy, compliance, drug-drug interactions, toxicity avoidance, and therapy cessation monitoring. Additionally, TDM can help to identify problems with medication compliance among noncompliant patient cases. Biologic medicinal products (biologics) have transformed treatment landscapes worldwide for patients with haematological or solid malignancies with the 21st century. Today, as data exclusivity periods of first wave biologics approach expiration/have expired, several biosimilar products (i.e., biologics that are considered to be similar in terms of quality, safety and efficacy to an approved 'reference' biologic) are being developed or have already been approved for human use. Like all biologics, biosimilars are structurally complex proteins that are typically manufactured using genetically engineered animal, bacterial or plant cell culture systems. As a consequence of this molecular complexity and the proprietary nature of the manufacturing process, which will inevitably result in the use of different host cell lines and expression systems as

well as related differences in manufacturing conditions, it is not possible to manufacture exact copies of a reference biologic. When administered to patients, all therapeutic proteins have the potential to induce an unwanted immune response (i.e., to stimulate the formation of antidrug antibodies [ADAs]). The impact of immune responses can range from no apparent effect to changes in pharmacokinetics, loss of effect and serious adverse events. Furthermore, the immunogenicity profile of a biologic can be significantly altered by even small differences in its manufacturing process that are accompanied by a change in product attributes, as well as differences in dosing schedules, administration routes or patient populations. Life Technologies® ELISA kits can be used for drug level and anti-drug antibodies measurements.

This Kit is based on Solid phase enzyme-linked immunosorbent assay (ELISA) in a sandwich format. Standards and samples (serum or plasma) are incubated in the microtiter plate coated with the drug filgrastim. After incubation, the wells are washed. Then, horse radish peroxidase (HRP) conjugated probe is added and binds to filgrastim antibodies captured by the drug filgrastim on the surface of the wells. Following incubation wells are washed and the bound enzymatic activity is detected by addition of tetramethylbenzidine (TMB) chromogen substrate. Finally, the reaction is terminated with an acidic stop solution. The colour developed is proportional to the amount of filgrastim antibodies in the sample or standard. Results of samples can be determined directly using the standard curve.

PRODUCT INFORMATION

MATERIALS SUPPLIED AND STORAGE CONDITIONS

Shipping at ambient temperature. Store kit reagents at 2-8 °C. Immediately after use remaining reagents should be returned to cold storage at 4 °C.

Microtiter Plate	1 x 12 x 8	Microtiter plate Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with filgrastim.
Standard A-F	0,3 mL (each)	Standards A-F (10x) Standard A: 500 ng/mL Standard B: 250 ng/mL Standard C: 125 ng/mL Standard D: 62,5 ng/mL Standard E: 31,2 ng/mL Standard F: 0 ng/mL Used for the standard curve. Contains antibodies to filgrastim, human serum and stabilizer, <0,1% NaN_3. Standards are prepared concentrated (10x). They should be diluted with the dilution rate given in the "Pre-test setup instructions" before the test.
Controls	0,3 mL (each)	Control low and high levels (10x) Contains human serum and stabilizer, <0,1% NaN_3. Controls are prepared concentrated (10x). They should be diluted with the dilution rate given in the "Pre-test setup instructions" before the test. Control concentrations are given in "Quality control certificate"
Assay Buffer	2 x 50 mL	Assay buffer Ready to use. Blue coloured. Contains proteins, <0,1% NaN_3.
Conjugate	1 x 12 mL	Horse radish peroxidase conjugated probe Ready to use. Red coloured. Contains HRP conjugated probe, stabilizer and preservatives.
Confirmation Reagent	1 x 1 mL	Confirmation reagent Ready to use. Contains proteins, filgrastim and stabilizer, 0,1% NaN_3
Substrate	1 x 12 mL	TMB substrate solution Ready to use. Contains 3,3',5,5'-Tetramethylbenzidine (TMB).
Stop Buffer	1 x 12 mL	TMB stop solution Ready to use. 1N HCl.
Wash Buffer	1 x 50 mL	Wash buffer (20x) Prepared concentrated (20x) and should be diluted with the dilution rate given in the "Pre-test setup instructions" before the test. Contains buffer with tween 20.
Foil	2 x 1	Adhesive Foil For covering microtiter plate during incubation

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator 2. Microplate reader 3. Precision pipettes and tips

4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

ASSAY PROTOCOL

SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines.

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8 °C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Note: Samples should be centrifuged adequately and no hemolysis or granule should be allowed.

REAGENT / PRE-TEST PREPARATIONS

Bring all reagents to room temperature before use. If crystals were formed in the Buffer Concentrates, warm them gently until they completely dissolved.

Wash buffer - Dilute with Distilled or deionized water 1:20.

Standards and Controls – Standards and controls to be diluted using Assay

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Buffer in dilution ratio of 1:10. For example 20µL standard/control + 180 µL assay buffer.

Serum / Plasma – Serum or Plasms to be diluted using Assay Buffer in dilution ratio of 1:1000. This can be done in two steps. In first step prepare 1:10 dilution e.g. 10µL sample + 90µL assay buffer. Subsequently, in second step prepare 1:100 dilution e.g. take 5µL of first step solution + 495µL assay buffer. Patient samples with a concentration above the measuring range are to be rated as > "Highest Standard (Standard A)". Such a patient sample in question should be further diluted with assay buffer and retested.

Confirmation Test Mixture – Mix diluted sample with Confirmation Reagent with Assay Buffer as diluent. For example, Preparing mixture: 10 µL diluted sample (1/1000) + 10 µL confirmation reagent + 100 µL assay buffer. Total volume: 120 µL.

ASSAY PROCEDURE

1. Dilute each of the standards, controls and samples as described.
2. Pipette 100 µL of each diluted “Standards”, “Low level control”, “High level control” and 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: Low level control ; H1: High level control ; A2 and on: Samples. Negative control can be duplicated to take the mean value.

3. Cover the plate with adhesive foil Briefly mix contents by gently shaking the plate Incubate 60 minutes at room temperature (18-25°C).
4. Remove adhesive foil Discard incubation solution Wash plate three times each

with 300 µL "Wash Buffer" Remove excess solution by tapping the inverted plate on a paper towel.

5. Pipette 100 µL "Conjugate" into each well.

6. Cover the plate with adhesive foil Incubate 60 minutes at room temperature (18-25°C).

7. Remove adhesive foil Discard incubation solution Wash plate three times each with 300 µL "Wash Buffer" Remove excess solution by tapping the inverted plate on a paper towel.

8. Pipette 100 µL "Substrate" into each well.

9. Incubate 20 mins without adhesive foil at room temperature (18-25°C) in dark.

10. Stop the substrate reaction by adding 100 µL "Stop Solution" into each well Briefly mix contents by gently shaking the plate Colour changes from blue to yellow.

11. Measure optical density with a photometer at OD 450nm with reference wavelength 650 nm (450/650 nm) within 30 minutes after pipetting the "Stop Solution.

CONFIRMATION TEST AND QC

Confirmation test for positive samples: Prepare confirmation test mixture as described earlier in Pre-test Preparations. Incubate this mixture 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. It is recommended to run only positive samples for confirmation testing. Negative samples may give improper results in confirmation step.

Quality Control: The test results are only valid if the test has been performed as

per the instructions. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. For a valid study, the OD 450/650 of the highest standard should be >1,500 and the OD 450/650 of the lowest standard should be reviewed.

CALCULATIONS

− Create a standard curve by using the standards. OD 450/650 nm for each standard on the vertical (Y-axis) axis versus the corresponding drug concentration on the horizontal (X-axis) axis.

− Standards and controls are prepared concentrated (10x). They should be diluted with the dilution rate given in the Pre-test setup instructions before the test. The standard curve should be prepared with the values obtained after dilution.

− 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 drug 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 drug concentration of the unknown sample.

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

− To obtain the exact values of the samples, the concentration determined from the standard-curve must be multiplied by the dilution factor (1000x). 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 determined 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 1/5 then results should be multiplied by 5000.

− For low and high level controls values, refer to Quality Control Certificate provided by each kit.

− Interpretation of true and false positives:

$$\frac{\text{OD 450/650 sample} - \text{OD 450/650 sample with confirmation reagent}}{\text{OD 450/650 sample}} \times 100 = \text{inhibition \%}$$

If the inhibition is ≥25% then the sample is "true positive". e.g.: If the OD 450/650 of the tested sample is 0.800 and after incubation of the sample with confirmation reagent and retested and the OD 450/650 of the sample is 0.200, then:

[(0.800-0.200)/0.800] X 100 = 75% i.e. sample is positive for anti-drug antibody.

− Qualitative interpretation: The results are evaluated by a cut-off value which is estimated by multiplying the mean OD 450/650 nm of the "standard F" by 3.

e.g. If "Sample OD 450/650 / the mean "standard F" OD 450/650 ≥ 3",

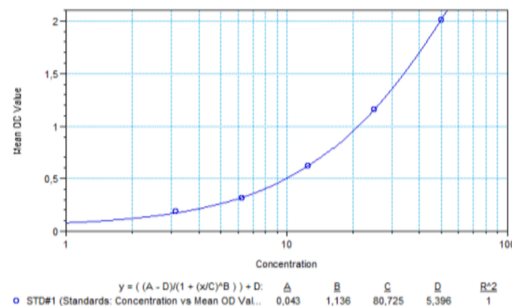
then the sample is POSITIVE

If "Sample OD 450/650 / the mean "standard F" OD 450/650,

then the sample is NEGATIVE

Note: The cut-off information provided with this kit can only be considered as a recommendation. Cut-off values must be calculated/set or verified according to scientific standards by the users/laboratories.

CHARACTERISTICS AND PERFORMANCE



− Calibration curve (Linearity, Dilutional linearity): $r^2 >0.95$

This is only an example. Assay conditions will change in every assay and do not use this curve for your assay calculations.

− Sensitivity: The lowest detectable level (Lowest detection limit, LOD) that can be distinguished from the zero standard is 3,1 ng/mL Functional sensitivity (Limit of quantification-LOQ): 3,1 ng/mL

− Specificity: There is no cross reaction with native serum immunoglobulin

− Recovery <100±30%.

− Precision: Intra-assay and inter-assay CVs <30%

− Cut-off: Cut-off values must be calculated/set or verified according to scientific standards by the users/laboratories. The Quality control certificate contains lot specific analytical performance data and is supplied separately with each kit.

PRECAUTIONS

1. Do not mix or substitute reagents with those from other lots or sources.
2. This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.
3. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
4. When mixing or reconstituting protein solutions, always avoid foaming.
5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
6. When using an automated plate washer, adding a 30 second soak period

following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

7. Stop Solution should be added to the plate in the same order as the Chromogen solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Chromogen solution.
8. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.

VALIDITY & STORAGE: 2-8°C, SEE KIT LABELS.

PLEASE REFER TO MANUAL SUPPLIED WITH KIT FOR INSTRUCTIONS FOR THE SPECIFIC BATCH SUPPLIED. THIS MANUAL IS FOR REFERENCE ONLY.

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