

Version 3c Last updated 12 April 2021

# ab237668 anti-Filgrastim ELISA Kit

For the measurement of antibody against Filgrastim in human serum and plasma.

This product is for research use only and is not intended for diagnostic use.

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# 1. Overview

anti-Filgrastim ELISA Kit (ab237668) is a highly specific and sensitive kit designed for the in vitro determination of antibody against Filgrastim in biological matrices such as human serum and plasma. The density of color is proportional to the amount of human antibody against Filgrastim captured from the samples.

Filgrastim is a recombinant, non-pegylated human granulocyte colony stimulating factor (G-CSF) analogue. It binds to the GCSF 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 is used in patients with acute myeloid leukemia receiving induction or consolidation chemotherapy. It is also used in cancer patients receiving bone marrow transplant. However, some patients develop unwanted immunogenicity, which leads to production of anti-drug-antibodies (ADAs) inactivating the therapeutic effects of the treatment and, in rare cases, inducing adverse effects.

## 2. Protocol Summary

Prepare all reagents, samples, and standards as instructed



Add 100  $\mu$ L standard, controls, diluted sample and confirmation test mixture to appropriate wells. Cover and incubate for 60 minutes at room temperature



Discard incubation solution and wash plate 3 times with 300  $\mu$ L diluted Wash Buffer



Add 100  $\mu$ L Peroxidase-conjugate to each well. Cover and incubate for 60 minutes at room temperature



Discard the solution and wash plate 3 times with 300  $\mu$ L diluted Wash Buffer.



Add 100  $\mu$ L TMB Substrate and incubate the plate in the dark at room temperature for 20 minutes.



Add 100  $\mu$ L Stop Solution and read OD at 450 nm within 20 minutes.

### 3. Precautions

**Please read these instructions carefully prior to beginning the assay.**

- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:  
[www.abcam.com/assaykitguidelines](http://www.abcam.com/assaykitguidelines)
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

### 4. Storage and Stability

**Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.**

Refer to list of materials supplied for storage conditions of individual components.

### 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors.

## 6. Materials Supplied

Item	Quantity	Storage condition
Micro ELISA Plate	1 unit	+4°C
Filgrastim Standard S1	300 µL	+4°C
Filgrastim Standard S2	300 µL	+4°C
Filgrastim Standard S3	300 µL	+4°C
Filgrastim Standard S4	300 µL	+4°C
Filgrastim Standard S5	300 µL	+4°C
Filgrastim Standard S6	300 µL	+4°C
Filgrastim Standard S7	300 µL	+4°C
Filgrastim Standard S8	300 µL	+4°C
Assay Buffer	2 x 50mL	+4°C
Peroxidase Conjugate	12 mL	+4°C
TMB substrate*	12 mL	+4°C
Stop Solution	12 mL	+4°C
Wash Buffer (20X)	50 mL	+4°C
Confirmation Reagent	1 mL	+4°C
Plate sealers	2 units	+4°C

\*Light sensitive. Store in the dark.

## 7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 450 nm
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for sample dilution.
- Plate shaker for all incubation steps.
- Absorbent paper

## 8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.

## 9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

### 9.1 20X Wash Buffer:

Dilute the 20X Wash Buffer to 1X solution in ddH<sub>2</sub>O (10 mL of Wash Buffer stock to 190 mL of ddH<sub>2</sub>O). Mix the 1X solution thoroughly by vortex manually. The working stock can be stable for 2 weeks after preparation at 4°C.

### 9.2 TMB substrate

Ready to use as supplied. Light sensitive. Store in the dark.

### 9.3 Peroxidase Conjugate

Ready to use as supplied.

### 9.4 Assay Buffer

Ready to use as supplied.

### 9.5 Stop Solution

Ready to use as supplied.



## 10. Standard and Control Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- Dilute the 10X stock solutions of standards with Assay (1: 10 dilution: 20 µL standard/control + 180 µL assay buffer)
- Please see table below for concentrations:

Name	S1	S2	S3	S4	S5	S6	S7	S8
Stock 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	-	-

Concentration for high and low controls are indicated on vials.

## 11. Sample Preparation

### General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.

### 11.1 Serum/plasma:

- 11.1.1 First dilute samples 1:10 (10 µL serum/plasma + 90 µL ddH<sub>2</sub>O) then 1:100 (5 µL diluted sample + 495 µL ddH<sub>2</sub>O) to get the final samples with dilution factor 1:1000.
- 11.1.2 Diluted samples should further be diluted if the concentration of Filgrastim is higher than the measuring range.
- 11.1.3 Samples are stable at 4°C for 7 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.

**Δ Note:** The usual precautions for venipuncture should be observed.

## 12. Confirmation test mixture preparation

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 at least 25% after incubation with the confirmation reagent.

## 13. Assay Procedure

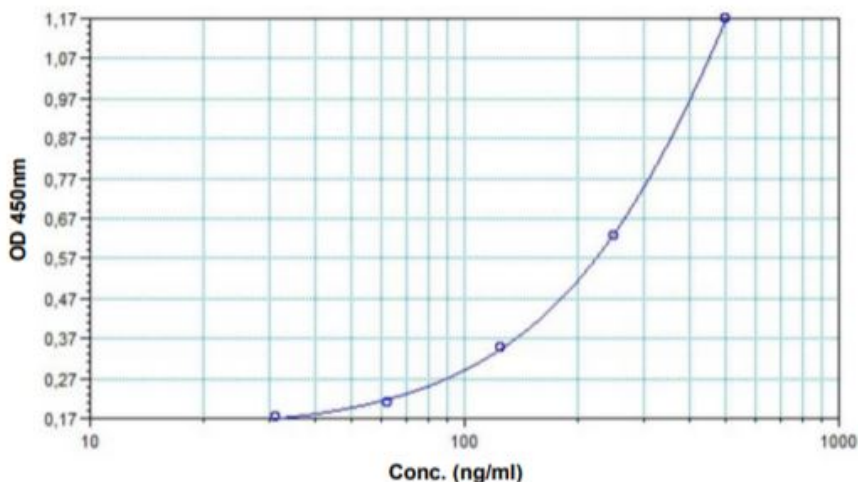
- Prepare reagents within 30 minutes before the experiment.
  - Equilibrate all materials and prepared reagents to room temperature 15 minutes prior to use.
  - We recommend that you assay all standards, controls and samples in duplicate.
  - A standard curve must be run with each assay.
- 
- 13.1** Add 100 µL of standards, controls, diluted samples and confirmation test mixture into appropriate wells. Cover wells and incubate for 60 minutes at room temperature.
  - 13.2** 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.
  - 13.3** Add 100 µL of Peroxidase conjugate into each well. Cover wells with adhesive plate sealer and incubate at room temperature for 60 minutes.
  - 13.4** Discard the solution and wash the wells as step 13.2.
  - 13.5** Add 100 µL of 1X TMB substrate solution and incubate the plate in the dark at room temperature for 20 minutes.
  - 13.6** Add 100 µL of Stop solution to stop the reaction.
  - 13.7** Read the absorbance in a microplate reader set to 450 nm within 20 minutes. (Reference wavelength to 650 nm).

## 14. Calculations

- 14.1 Calculate the average absorbance value for the blank control (0 ng/mL) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- 14.2 **Create a standard curve** by plotting the average blank control subtracted absorbance value for each standard concentration (y axis) against the target protein concentration (x axis) of the standard.
- 14.3 Construct a standard curve of difference data using software capable of generating four-parameter logistic (4PL) or point-to-point calculation curve fit.
- 14.4 To obtain the exact values of the samples, the concentration determined from the standard curve should be multiplied by the dilution factor.
- 14.5 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted **absorbance values against the standard curve**. Multiply the resulting value by the appropriate dilution factor to obtain the concentration of target protein in the sample.
- 14.6 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

## 15. Typical Data

Typical standard curve - data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



**Figure 1.** Typical Standard Curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

## 16. Typical Sample Values

**Detection Range:** 3.1 - 50 ng/mL.

**Sensitivity:** 3.1 ng/mL.

**Assay Precision:** Intra-Assay: CV < 30%; Inter-Assay: CV < 30% (CV (%) = SD/mean X 100)

**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.

**Recovery rate:** <100±30% with normal human serum samples with known concentrations.

## 17. Notes



## Technical Support

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