ab325033 – Rat GCSF ELISA Kit (RUO)

For the quantitative determination of GCSF in rat serum.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab325033

Storage and Stability: Store the whole kit at 2-8°C immediately upon receipt. Please refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent preparation sections.

Materials Supplied

Item	Quantity 1 x 96 tests	Storage
GCSF Protein Coated Microtiter Plate (12x8 wells)	1 unit	2-8°C
Anti-GCSF Standard, (0.5ml/vial)	2 vials	2-8°C
GCSF Protein: HRP Conjugate	12 mL	2-8°C
(1X) Sample Diluent	2 x 50 mL	2-8°C
(1X) Standard Diluent	10 mL	2-8°C
(20X) Wash Buffer	25 mL	2-8°C
TMB Substrate	12 mL	2-8°C
Stop Solution	12 mL	2-8°C

Materials Required, Not Supplied

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

- Microtiter Plate Reader able to measure absorbance at 450 nm
- Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 µL to 1000 µL
- Deionized (DI) water
- Wash bottle or automated microplate washer
- Graph paper or software for data analysis
- Timer
- Absorbent Paper

Handling/Storage

- All reagents should be stored at required temperature as indicated on the component label.
- All the reagents and wash solutions should be used within 12 months from manufacturing date.
- Before using, bring all components to room temperature (18-25°C). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
- The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Sample Preparation and Storage

- Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20°C.

Preparation Before Use

- Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.
- Serum Test Sample preparation - Samples have to be diluted 1:1000 (v/v), e.g. 1 µL sample + 999 µL sample diluent) prior to assay. The samples may be kept at 2-8°C for up to three days. Long-term storage requires -20°C.

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

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make (1X) Wash Buffer: dilute 25 mL of (20X) Wash Buffer in 475 mL of DI water.

Standards Preparation

1. Reconstitute the concentrated Standard lyophilized vial with 1 mL of Standard Diluent to obtain a concentration of 1 µg/mL. Keep the vial for 15 minutes with gentle agitation before making further dilutions.
2. Dilute 320 µL of original Standard (1 µg/mL) with 180 µL of Standard Diluent to generate a 640 ng/mL Standard Solution.
3. Prepare further Standards by serially diluting the Standard Solution as per the below table. Use the Standard Diluent as the Zero Standard (Standard #0).

∆***Note:*** *Use the Standards as soon as possible upon reconstitution. Discard balance standard after use.*

Standard Concentration	Standard Vial	Dilution Particulars
1 µg/mL	Original Standard	Lyophilized Standard provided in the Kit + 1 mL Standard Diluent (1X)
640 ng/mL	Standard#7	320 µL Reconstituted Standard (1 µg/mL) + 180 µL Standard Diluent (1X)
320 ng/mL	Standard#6	250 µL Standard#7 + 250 µL Standard Diluent (1X)
160 ng/mL	Standard#5	250 µL Standard#6 + 250 µL Standard Diluent (1X)
80 ng/mL	Standard#4	250 µL Standard#5 + 250 µL Standard Diluent (1X)
40 ng/mL	Standard#3	250 µL Standard#4 + 250 µL Standard Diluent (1X)
20 ng/mL	Standard#2	250 µL Standard#3 + 250 µL Standard Diluent (1X)
10 ng/mL	Standard#1	250 µL Standard#2 + 250 µL Standard Diluent (1X)
0 ng/mL	Standard#0	Only Standard Diluent (1X)

Procedural Notes

- In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
- High Dose Hook Effect may be observed in samples with very high concentrations of GCSF protein. High Dose Hook Effect is due to excess of antibody for very high concentrations of GCSF protein present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus, if the Anti-Teriparatide concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
- Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Liraglutide.
- It is recommended that the Standards and Samples be assayed in duplicates.
- Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
- If the Substrate has a distinct blue color prior to use it may have been contaminated, and use of such substrate can lead to the sensitivity of the assay being compromised.
- The plates should be read within 30 minutes after adding the Stop Solution.
- Make a work list in order to identify the location of Standards and Samples.

Assay Procedure

- It is strongly recommended that all Standards and Samples be run in duplicates or triplicates.
- A standard curve is required for each assay.
- All steps must be performed at 37°C.

1. Add 100 µL of prepared Standards or diluted Samples into the respective wells.
2. Cover the plate and incubate for 60 minutes at 37°C.
3. Aspirate and wash plate 4 times with (1X) Wash Buffer 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.
4. Pipette without delay in the same order 100 µL of GCSF Protein: HRP Conjugate into each well.
5. Cover the plate and incubate for 60 minutes at 37°C.
6. Aspirate and wash plate 4 times with (1X) Wash Buffer 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.
7. Add 100 µL of TMB Substrate in each well.
8. Incubate the plate at 37°C for 30 minutes in dark. **DO NOT SHAKE** or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
9. Pipette out 100 ul of Stop Solution. Wells should turn from blue to yellow in color.
10. Read the absorbance at 450 nm with a microplate reader.

Calculation of Results

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using graph paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown GCSF protein concentrations, find the unknown's 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 GCSF protein Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit, or a polynomial curve (2nd order) is best recommended for automated results.

∆***Note:*** *It is recommended to repeat the assay at a different dilution factor if the sample absorbance value is below the first standard or if the absorbance value is equivalent or higher than the 640 ng/ml standard.*

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide
For technical support contact information, visit: www.abcam.com/contactus

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