User Guide

Human GMCSF R alpha ELISA Kit

For serum, plasma, and cell culture supernatants

RAB1487

Introduction

The Human GMCSF R alpha ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human GMCSF R alpha in serum (human GMCSF R alpha concentration is pretty low in normal serum/plasma, it may not be detected in this assay), plasma and cell culture supernatants. This assay employs an antibody specific for human GMCSF R alpha coated on a 96-well plate. Standards and samples are pipetted into the wells and GMCSF R alpha present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human GMCSF R alpha antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of GMCSF R alpha bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Storage

The entire kit may be stored at -20°C for up to 1 year from date of shipment. Avoid repeated freeze thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.

Components

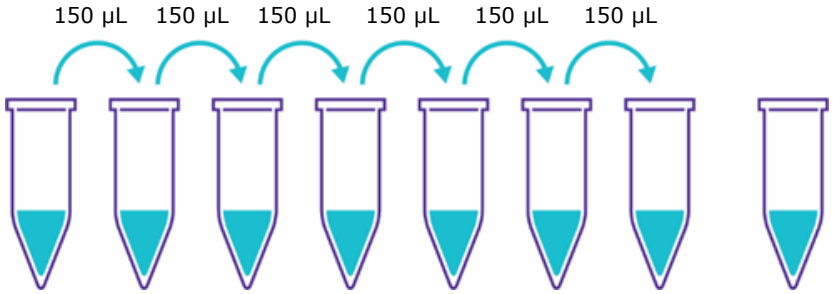
- Human GMCSF R alpha Microplate - RAB1487A-1EA: 96 wells (12 strips x 8 wells) coated with anti-Human GMCSF R alpha. Stable in storage 1 month at 4 °C once opened. Return unused wells to the pouch containing desiccant pack, reseal along the edge.
- Human GMCSF R alpha Standard Protein - RAB1487C-1VL: 2 vials of Human GMCSF R alpha. 1 vial is enough to run each standard in duplicate. Stable in storage 1 week at -80 °C once opened.
- Human GMCSF R alpha Detection Antibody - RAB1487F-1VL: 2 vials of biotinylated anti-Human GMCSF R alpha. Each vial is enough to assay half the microplate. Stable in storage 5 days at 4 °C once opened.
- Wash Buffer - RABWASH4: 25 mL of 20X concentrated solution. Stable in storage 1 month at 4 °C once opened.
- HRP-Streptavidin - RABHRP5: 200 µL 300X concentrated HRP-conjugated streptavidin. Do not store and reuse.
- TMB One-Step Substrate Reagent - RABTMB3: 12 mL of 3,3',5,5'-tetramethylbenzidine (TMB) in buffer solution.
- Stop Solution - RABSTOP3: 8 mL of 0.2 M sulfuric acid.
- 5X Assay Diluent D - RABELADD-15ML: 15 mL of 5X concentrated buffer. Stable in storage 1 month at 4°C once opened.
- 5X Assay Diluent B - RABELADE-15ML: 15 mL of 5X concentrated buffer. Stable in storage 1 month at 4°C once opened.

Additional Materials Required (But Not Provided)

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µl to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and l liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

Reagent Preparation

1. Bring all reagents and samples to room temperature (18-25 °C) before use.
2. 5X Assay Diluent D and 5X Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
3. Sample dilution: 1X Assay Diluent D should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2-fold.
   Note: Levels of GMCSF R alpha may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.
4. Preparation of standard: Briefly spin a vial of Standard Protein. Add 400 µL 1X Assay Diluent D (should be diluted 5-fold with deionized or distilled water before use) into the Standard Protein vial to prepare a 200 ng/mL standard solution. Dissolve the powder thoroughly by a gentle mix. Pipette 300 µL 1X Assay Diluent D into each tube. Use the 200 ng/mL standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1X Assay Diluent D serves as the zero standard (0 ng/mL).



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	0 Std
Diluent volume	Std + 400 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL
Concentration	200 ng/mL	66.67 ng/mL	22.22 ng/mL	7.407 ng/mL	2.469 ng/mL	0.823 ng/mL	0.274 ng/mL	0 ng/mL

5. If the Wash Buffer (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.
6. Briefly spin the Detection Antibody vial before use. Add 100 µL of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B and used in step 5 of the assay procedure.
7. Briefly spin the HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 300-fold with 1X Assay Diluent B.

   For example: Briefly spin the HRP-Streptavidin vial and pipette up and down to mix gently. Add 40 µL of HRP-Streptavidin concentrate into a tube with 12 mL 1x Assay Diluent B to prepare a 300-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Assay Procedure

1. Bring all reagents and samples to room temperature (18-25 °C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Label removable 8-well strips as appropriate for your experiment.
3. Add 100 µL of each standard (see Reagent Preparation step 4) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.
4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of 1X prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
6. Discard the solution. Repeat the wash as in step 4.
7. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
8. Discard the solution. Repeat the wash as in step 4.
9. Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
10. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

Assay Procedure Summary

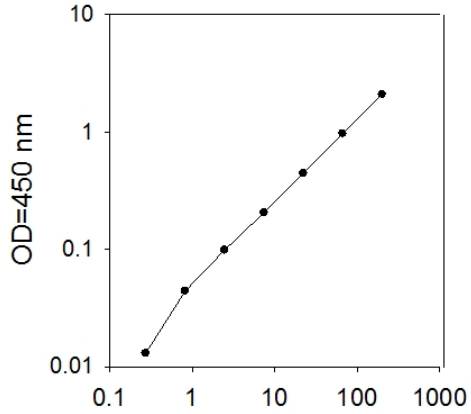
1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µL standard or sample to each well. Incubate 2.5 hours at room temperature.
3. Add 100 µL prepared biotin antibody to each well. Incubate 1 hour at room temperature.
4. Add 100 µL prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µL TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 µL Stop Solution to each well. Read at 450 nm immediately.

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.



Sensitivity

The minimum detectable dose of Human GMCSF R alpha was determined to be 0.3 ng/mL..

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

Spiking & Recovery

Recovery was determined by spiking various levels of Human GMCSF R alpha into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	84.13	73-97
Plasma	76.13	67-85
Cell culture media	76.57	67-86

Linearity

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	78.12	96.65	90.92
	Range (%)	68-88	68-147	69-113
1:4	Average % of Expected	74.10	101.9	132.3
	Range (%)	71-77	72-149	127-137

Reproducibility

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

Specificity

This ELISA kit shows no cross-reactivity with the following cytokines tested: human B7-2 (CD86); BAFF R (TNFRSF13C); Calcitonin; Calsyntenin-1; Cathepsin E; cIAP-2 (HIAP-1); Coagulation Factor VII; Complement MASP3; Endocan; EphA2; EphB4; Ephrin-A4; FGF-23; FGF-5; Flt-3 (Flk-2); GLP-1 (7-37 & 7-36-NH2); Glypican 2; GP73 (GOLM1); HTRA2 (Omi); IL-20 R alpha; IL-4 R alpha; JAM-C; Luteinizing Hormone (LH); Matrilin-3; Meprin alpha (MEP1A); MSP R (Ron); N-Cadherin; Neprilysin-2 (MMEL1); NKp44; Pappalysin-1 (PAPP-A); Parathyroid hormone (PTH); Pepsinogen II (PGC); Peptide YY (PYY); Presenilin 1 (PSEN1); SOX2; TFPI-2; TRACP (ACP5); Trefoil factor 3 (TFF3); Ubiquitin+1.

Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Briefly centrifuge the standard protein and dissolve the powder thoroughly by gently mixing.
Low signal	Improper preparation of standard and/or biotinylated antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly.
	Too brief incubation times	Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4 °C with gentle shaking. Note: may increase overall signals including background.
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
	Air bubblies in wells	Remove bubbles in wells
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your standard at -70 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light.
	Stop Solution	Add stop solution to each well before reading plate

Notice

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