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ab267573
Mouse GITRL ELISA Kit

For the quantitative measurement of mouse GITRL in cell culture supernatants, plasma and serum.

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

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1. Overview

Mouse GITRL ELISA Kit is designed for the quantitative determination of GITRL in cell culture supernatants, plasma and serum samples.

This assay employs an antibody specific for mouse GITRL coated on a 96-well plate. Standards and samples are pipetted into the wells and GITRL present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse GITRL 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 GITRL bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed.

Add 100 µL standard or sample to each well. Incubate 2.5 hours at room temperature.

Add 100 µL prepared biotin antibody to each well. Incubate 1 hour at room temperature.

Add 100 µL prepared Streptavidin solution. Incubate 45 minutes at room temperature.

Add 100 µL TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

Add 50 µL Stop Solution to each well. Read at 450 nm immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All ELISA kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle 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 pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- 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

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

Observe the storage conditions for individual prepared components in the Reagent Preparation section 9.

5. Limitations

- ELISA 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. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
200X HRP-Streptavidin Concentrate	200 µL	-20°C
Wash Buffer (20X)	25 mL	-20°C
Assay Diluent A	30 mL	-20°C
5X Assay Diluent B	15 mL	-20°C
Stop Solution	8 mL	-20°C
TMB Substrate Solution	12 mL	-20°C
Biotinylated Anti-Mouse GITRL Detection Antibody	2 vials	-20°C
Anti-Mouse GITRL coated Microplate (12 x 8 wells)	1 unit	-20°C
Mouse GITRL Standard (Lyophilized)	2 vials	-20°C

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 450 nm.
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution or sample dilutions.
- 100 mL and 1 L graduated cylinders.
- Protease and phosphatase inhibitors.

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- 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.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 Assay Diluent A:

Ready to use as supplied.

9.2 Assay Diluent B:

Dilute 5X Assay Diluent B 5-fold with deionized or distilled water before use.

9.3 Biotinylated Anti-Mouse GITRL Detection Antibody:

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.

9.4 20X Wash Buffer:

If the Wash Concentrate (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.

9.5 200X HRP-Streptavidin Concentrate:

Briefly spin the vial of HRP-Streptavidin concentrate before use. HRP-Streptavidin should be diluted 200-fold with 1X Assay Diluent B.

For example: Briefly spin the vial and pipette up and down to mix gently. For example: Add 50 µL of HRP-Streptavidin concentrate into a tube with 10 mL 1X Assay Diluent to prepare a 200-fold diluted HRP-Streptavidin solution (do not store the diluted solution for next day use). Mix well.

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10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Briefly spin the Standard Vial.

10.2 Add 400 µL of Assay Diluent A (for plasma/serum samples) or 1X Assay Diluent B (for cell culture supernatant samples) into Standard Vial to prepare a 50 ng/mL Standard solution. Gently mix the powder to allow it to dissolve thoroughly. If a precipitate is seen in the solution after mixing, this can be removed by a quick centrifuge of the standard vial, and then pipetting the supernatant only for the assay.

10.3 Pipette 20 µL of the 50 ng/mL Standard into 980 µL Assay Diluent A or 1X Assay Diluent B to generate a 1000 pg/mL Standard solution.

10.4 Pipette 300 µL of Assay Diluent A or 1X Assay Diluent B into each tube.

10.5 Use the Standard solution to produce a dilution series. Adding 200 µL from #1 to #2, then from #2 to #3, etc.

10.6 Mix each tube thoroughly before the next transfer.

10.7 Tube #8 contains no protein and is the Blank control.

Tube #	Volume to dilute	Volume of Assay Diluent A or B	Final Concentration pg/mL
1	1000 pg/mL Standard Solution	---	1000
2	200 µL of tube #1	300 µL	400
3	200 µL of tube #2	300 µL	160
4	200 µL of tube #3	300 µL	64
5	200 µL of tube #4	300 µL	25.6
6	200 µL of tube #5	300 µL	10.24
7	200 µL of tube #6	300 µL	4.1
8	---	300 µL	0

11. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

ΔNote: Assay Diluent A should be used for dilution of serum and plasma samples. 1X Assay Diluent B should be used for dilution of cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2-fold.

11.1 Label removable 8-well strips as appropriate for your experiment.

11.2 Add 100 µL of standard or sample into appropriate wells. Cover the wells and incubate for 2.5 hours at room temperature with gentle shaking.

11.3 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 auto-washer. Complete removal of liquid at each step is essential for 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.

11.4 Add 100 µL of 1X prepared biotinylated antibody to each well. Incubate for 1 hour at room temperature with gentle shaking.

11.5 Discard the solution. Repeat the wash as in step 11.3.

11.6 Add 100 µL of prepared Streptavidin solution each well. Incubate for 45 minutes at room temperature with gentle shaking.

11.7 Discard the solution. Repeat the wash as in step 11.3.

11.8 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.

11.9 Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

12. Calculations

12.1 Calculate the mean absorbance for each set of duplicate standards, controls and samples.
12.2 Subtract the average zero standard optical density.
12.3 Plot the standard curve on log-log, with standard concentration on the x-axis and absorbance on the y-axis.
12.4 Draw the best-fit straight line through the standard points.

13. Typical Data

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

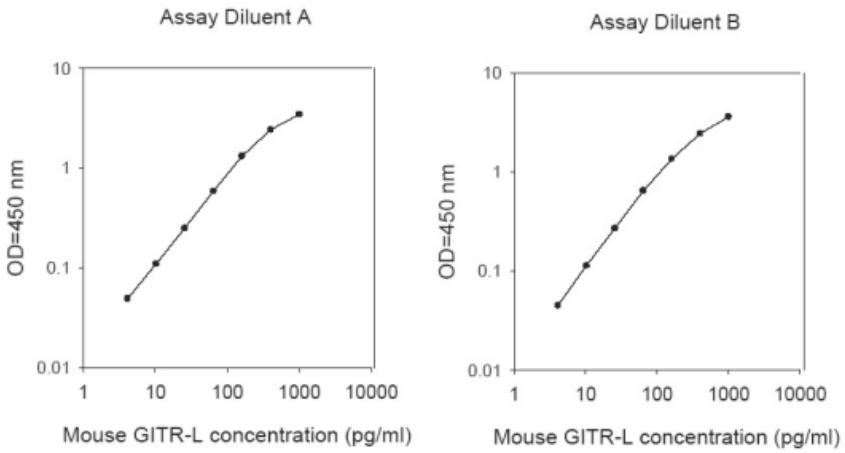

Figure 1. Mouse GITRL ELISA kit (ab267573) Standard curves.

Sensitivity:

The minimum detectable dose of mouse GITRL was determined to be 4 pg/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).

Reproducibility:

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

Spiking and Recovery:

Recovery was determined by spiking various levels of Mouse GITRL into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % recovery	Range %
Serum	99.4	91-107
Plasma	98.14	91-103
Cell culture media	105.3	97-113

Linearity:

Sample Type		Serum	Plasma	Cell culture media
1:2	Average % of expected	118.9	123.8	101.8
	Range %	111-127	115-132	94-110
1:4	Average % of expected	124.5	140	80.92
	Range %	117-133	131-148	73-89

Specificity:

This ELISA kit shows no cross-reactivity with the following cytokines tested: Mouse CD30L, CD30, CD40, CRG-2, CTACK, CXCL16, Eotaxin , Eotaxin-2, Fas Ligand, Fractalkine, GCSF, GM-CFS, IFN-gamma, IGFBP-3, IGFBP-5, IGFBP-6, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-3 Rb, IL-4, IL-5, IL-9, IL-10, IL-12 p40/p70, IL-12 p70, IL-13, IL-17, KC, Leptin R, LEPTIN(OB), LIX, L-Selectin, Lymphotactin, MCP-1, MCP-5, M-CSF, MIG, MIP-1 alpha, MIP-1 gamma, MIP-2, MIP-3 beta, MIP-3 alpha, PF-4, P-Selectin, RANTES, SCF, SDF-1 alpha, TARC, TCA-3, TECK, TIMP-1, TNF-alpha, TNF RI, TNF RII, TPO, VCAM-1, VEGF.

14. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle 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. Sample and standard addition 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 bubbles 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

15. Notes

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