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ab100689 Interferon gamma (IFNG) Mouse ELISA Kit

For the quantitative measurement of Interferon gamma in Mouse serum, plasma and cell supernatants.

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

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1. Overview

Abcam's Interferon gamma Mouse ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of Mouse Interferon gamma in serum, plasma and cell culture supernatants.

This assay employs an antibody specific for Mouse Interferon gamma coated on a 96- well plate. Standards and samples are pipetted into the wells and Interferon gamma present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-Mouse Interferon gamma 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 Interferon gamma 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 standard or sample to each well used. Incubate at room temperature.

Add prepared biotin antibody to each well. Incubate at room temperature.

Add prepared Streptavidin solution. Incubate at room temperature.

Add TMB One-Step Development Solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read at 450nm immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All 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 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.
- 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 -20°C immediately upon receipt. Avoid multiple freeze-thaw cycles. 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. Observe the storage conditions for individual prepared components in the Materials Supplied section.

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. 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
Interferon gamma Microplate (12 x 8 wells)	96 wells	-20°C
20X Wash Buffer	25 mL	-20°C
Recombinant Mouse Interferon gamma Standard	2 vials	-20°C
Assay Diluent A	30 mL	-20°C
5X Assay Diluent B	15 mL	-20°C
Biotinylated anti-Mouse Interferon gamma	2 vials	-20°C
440X HRP-Streptavidin Concentrate	200 µL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C
Stop Solution	8 mL	-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.
- Precision pipettes to deliver 2 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 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.

8. Technical Hints

- 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.
- 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.
- When preparing your standards, it is very critical to briefly spin down the vial first. The powder may drop off from the cap when opening it if you do not spin down. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and then spin it down; repeat this procedure 3-4 times. This is a technique we find very effective for thoroughly mixing the standard without too much mechanical force.
- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.
- Keep the standard dilutions on ice while during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.

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 1X Assay Diluent B

5X Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.

9.2 1X Wash Solution

If the 20X Wash Concentrate contains visible crystals, equilibrate to room temperature and mix gently until dissolved. Dilute 20 mL of 20X Wash Buffer into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

9.3 1X Biotinylated Interferon gamma Detection Antibody

Briefly spin the Biotinylated anti-Mouse Interferon gamma 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 either be stored at 4°C for 5 days or aliquoted and frozen at -20°C for 2 months). The detection antibody concentrate must be diluted 120-fold with 1X Assay Diluent B prior to use in the Assay Procedure.

9.4 1X HRP-Streptavidin Solution

Briefly spin the 440X HRP-Streptavidin concentrate vial before use. HRP-Streptavidin concentrate must be diluted 440-fold with 1X Assay Diluent B prior to use in the Assay Procedure.

For example: Briefly spin the vial and pipette up and down to mix gently. Add 25 µL of 440X HRP-Streptavidin concentrate into a tube with 11 mL 1X Assay Diluent B to prepare a final 440-fold diluted 1X HRP-Streptavidin solution. Mix well.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.
- Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution.

10.1 Briefly spin the vial of Interferon gamma Standard. Prepare a 20 ng/mL Interferon gamma **Stock Standard** by adding 400 µL Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture supernatants) into the vial (see table below).

10.2 Dissolve the powder thoroughly by gentle mixing.

10.3 Label tubes #1-7.

10.4 Prepare Standard #1 by adding 50 µL 20 ng/mL Stock Standard to 450 µL of Assay Diluent A or 1X Assay Diluent B into tube #1. Mix thoroughly and gently.

10.5 Pipette 400 µL Assay Diluent A or 1X Assay Diluent B into each tube.

10.6 Prepare Standard #2 by transferring 200 µL from tube #1 to #2, mix thoroughly.

10.7 Prepare Standard #3 by transferring 200 µL from tube #2 to #3, mix thoroughly.

10.8 Using the table below as a guide, prepare further serial dilutions.

10.9 Assay Diluent A or 1X Assay Diluent B serves as the zero standard (0 pg/mL).

Standard #	Volume to dilute (µL)	Volume Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	50	450	20,000	2,000
2	200 µL Standard #1	400	2,000	666.7
3	200 µL Standard #2	400	666.7	222.2
4	200 µL Standard #3	400	222.2	74.07
5	200 µL Standard #4	400	74.07	24.69
6	200 µL Standard #5	400	24.69	8.23
7	200 µL Standard #6	400	8.23	2.74
8	-	400	0	0

11. Sample Preparation

General Sample Information:

- If your samples need to be diluted, 1X Assay Diluent B should be used for dilution of culture supernatants. Assay Diluent A should be used for dilution of serum/plasma samples.
- Suggested dilution range for normal serum/plasma: 2-10-fold.
- Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. 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.

13.1 Add 100 µL of each standard (see Standard Preparation section 10) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.

13.2 Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with 1X Wash Solution (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 1X Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

13.3 Add 100 µL of 1X Biotinylated Interferon gamma Detection Antibody (Reagent Preparation section 9) to each well. Incubate for 1 hour at room temperature with gentle shaking.

13.4 Discard the solution. Repeat the wash as in step 13.2.

13.5 Add 100 µL of 1X HRP-Streptavidin solution (see Reagent Preparation section 9) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

13.6 Discard the solution. Repeat the wash as in step 13.2.

13.7 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.

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

14. Calculations

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, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

15. Typical Data

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

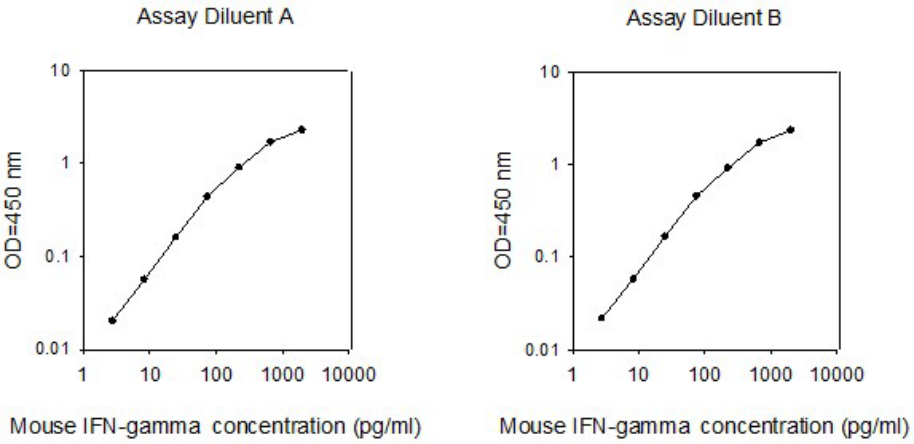

Figure 1. Example of Mouse Interferon gamma standard curve.

Conc. (pg/mL)	O.D.	
	Assay Diluent A	Assay Diluent B
2.74	0.014	0.013
8.23	0.04	0.037
24.69	0.13	0.125
74.07	0.354	0.349
222.2	0.946	0.94
666.7	2.048	1.989
2,000	2.93	2.9

16. Typical Sample Values

SENSITIVITY –

The minimum detectable dose of Interferon gamma was determined to be 1.3 pg/mL.

RECOVERY –

Recovery was determined by spiking various levels of Interferon gamma into normal Mouse serum. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	95.38	83-103
Plasma	93.49	82.102
Cell Culture Media	94.75	83-103

LINEARITY OF DILUTION –

Serum Dilution	Average % Expected Value	Range (%)
1:2	90	82-103
1:4	94	84-104

Plasma Dilution	Average % Expected Value	Range (%)
1:2	92	83-103
1:4	93	84-104

Cell Culture Media Dilution	Average % Expected Value	Range (%)
1:2	91	82-102
1:4	94	85-104

PRECISION –

	Intra-Assay	Inter-Assay
CV (%)	<10%	<12%

17. Assay Specificity

CROSS REACTIVITY –

This ELISA kit shows no cross-reactivity with any of the cytokines tested (e.g., Mouse CD30, L CD30, T CD40, CRG-2, CTACK, CXCL16, Eotaxin , Eotaxin-2, Fas Ligand, Fractalkine, GCSF, GM-CFS, IGFBP-3, IGFBP-5, IGFBP-6, IL-1 a, IL-1β, IL-2, IL-3, IL-3 Rb, IL-4, IL-5, IL-6, 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-1a, MIP-1γ, MIP-2, MIP-3β, MIP-3a, PF-4, P-Selectin, RANTES, SCF, SDF-1a, TARC, TCA-3, TECK, TIMP-1, TNF-a,TNFRI,TNFRII,TPO,VCAM-1,VEGF).

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

19. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)