

Mouse Anti-Human Interferon gamma (IFN- γ) ELISA Set

Cat. No.	Form	Quantity
15600-01	Set	1

Intended Use

This set is specifically designed for quantitative determination of human interferon gamma (IFN- γ) concentrations in cell culture supernatant, serum and plasma.

Background

Human interferon gamma (IFN- γ) is a 20 or 25 kD glycoprotein that is secreted by a variety of cells, including T lymphocytes and natural killer (NK) cells¹. This sole member of the type II interferon family is structurally and functionally distinct from type I members, including IFN- α and IFN- β ². IFN- γ production is primarily regulated via cytokine secretion by antigen-presenting cells (APCs)^{3,4}. Pathogen-induced activation of APCs results in secretion of interleukin-12 and interleukin-18 that not only attract T lymphocytes and NK cells to the site of inflammation, but also induce these cells to produce IFN- γ . This up-regulation of IFN- γ promotes receptor-mediated activation of the Jak-Stat signaling pathway in target cells, leading to transcriptional modifications of target genes via specific response elements⁵⁻⁷. IFN- γ mediates numerous functions in the inflammatory process. It has been shown to orchestrate specific immune cell trafficking to sites of inflammation by stimulating production of cell adhesion molecules and chemokines^{3,8}. It promotes host response to intracellular pathogens by up-regulating cell-surface class I and II MHC molecules^{9,10}. IFN- γ also activates microbicidal effector functions in macrophages by increasing pinocytosis and receptor-mediated phagocytosis in these cells¹¹. Furthermore, studies have shown that IFN- γ may coordinate the transition from innate immunity to adaptive immunity by promoting a Th1-type response and enhancing B cell isotype switching to IgG2a^{12,13}.

Principle of Assay

This assay incorporates a quantitative sandwich enzyme immunoassay technique. Unlabeled monoclonal antibody specific for human interferon gamma (IFN- γ) is first coated onto 96 well microplate(s). Standards and samples are then added to the wells and any IFN- γ present is captured by the immobilized antibody. After a wash to remove unbound material, biotin-labeled anti-human IFN- γ detection antibody is added to the wells, followed by a horseradish peroxidase-labeled streptavidin incubation. After another wash, TMB substrate solution is added that will result in a blue color proportional to the amount of bound IFN- γ . Color development is then quenched and intensity is measured at 450nm.

Antibody Description

Antigen Source: Human
Immunogen: Interferon gamma (IFN- γ)
Classification: Inflammation marker

Capture Antibody

Host: Mouse
Clone: A35
Isotype: IgG1

Detection Antibody

Host: Mouse
Clone: B27
Isotype: IgG1

Research Applications

Enzyme-Linked-Immunosorbent-Assay (ELISA)

Assay Kit Components

- 0.3ml IFN- γ Capture Antibody: unlabeled, 200X
- 0.06ml IFN- γ Detection Antibody: biotin-conjugated, 1000X
- 0.3ml Detection Enzyme: HRP-conjugated, 200X
- 2 vials Standard: lyophilized recombinant human IFN- γ , 2.5ng/vial
- 15ml 5X Coating Buffer
- 300ml Assay Diluent (1X)
- 300ml Washing Buffer (20X)
- 60ml Standard/Sample Diluent
- 60mL TMB one component microwell substrate
- 60mL TMB Stop Solution
- 5 x 96-well ELISA plates

REAGENT PREPARATION

All reagents should be diluted immediately prior to use. Do not add sodium azide to Assay Diluent as it inhibits the activity of horseradish-peroxidase. It is recommended that all samples, controls and standards be assayed in duplicate or triplicate.

- Dilute 5X Coating Buffer to 1X with distilled (DI) water. A suggested dilution (for one plate) consists of 2.2mL 5X Coating Buffer with 8.8mL of DI water.
- Dilute unlabeled human IFN- γ specific Capture Antibody 200-fold with 1X Coating Buffer. A suggested dilution (for one plate) consists of 55 μ L Capture Antibody with 10.945mL 1X Coating Buffer.
- Dilute 20X Wash Buffer to 1X with DI water. A suggested dilution consists of 50mL 20X Wash Buffer with 950mL of DI water.
- Reconstitute 1 vial lyophilized human IFN- γ Standard by adding 0.1ml DI water to 1 vial (25ng/mL) and gently vortex. Dilute IFN- γ Standard 100-fold by adding 5 μ L 25ng/mL Standard to 495 μ L Standard/Sample diluent to yield a final concentration of 250pg/mL and allow the standard to equilibrate for at least 15 minutes. Aliquot any unused standard into polypropylene vials and store at -80°C.
- Dilute biotin-labeled human IFN- γ specific Detection Antibody 1000-fold with 1X Assay Diluent. A suggest dilution (for one plate) consists of 11 μ L Detection Antibody with 10.989mL 1X Assay Diluent.
- Dilute horseradish peroxidase-labeled Detection Enzyme 200-fold with 1X Assay Diluent. A suggested dilution (for one plate) consists of 55 μ L Detection Enzyme with 10.945mL 1X Assay Diluent.

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Assay Procedure

1. Collect samples and prepare reagents as directed previously.
2. Add 100µL of diluted Capture Antibody solution to all wells of a provided 96-well plate. Incubate at 4°C overnight.
3. Wash entire plate 4 times with 1X Wash Buffer.
4. Add 300µL of 1X Assay Diluent per well to block non-specific binding and reduce background. Incubate at room temperature for 30 minutes.
5. Wash plate as in Step 3.
6. Prepare standard and sample dilutions and add 100µL/well of appropriate dilution to the plate. To do this, perform six two-fold serial dilutions of the 250pg/mL top standard either within the plate or in separate tubes. To dilute within the plate, add 200µl of 250pg/mL human IFN-γ Standard to row A and 100µl Standard/Sample diluent to rows B through H. Perform serial dilution by taking 100µl standard from row A and mix with row B (see below). Then take 100µl from row B and add it to row C and continue for each subsequent row. Therefore, the final human IFN-γ standard concentrations will be 250pg/mL, 125pg/mL, 62.5pg/mL, 31.3pg/mL, 15.6pg/mL, 7.8pg/mL and 3.9pg/mL. Standard/Sample Diluent alone serves as the zero standards for row H (0pg/mL). Incubate at room temperature for 2 hours.
7. Wash plate as in Step 3.
8. Add 100µL of diluted biotin-labeled Detection Antibody solution to each well and incubate at room temperature for 1 hour.
9. Wash plate as in Step 3.
10. Add 100µL of diluted HRP-labeled Detection Enzyme solution to each well and incubate at room temperature for 30 minutes.
11. Wash plate 6 times with 1X Wash Buffer. For this final wash, soak wells in Wash Buffer for 10 seconds for each wash. This will help minimize background.
12. Add 100µL of TMB Substrate Solution to each well. Incubate at room temperature for 10-15 minutes. The Substrate Solution should turn blue in color.
13. Add 50µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells turns green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
14. Determine the optical density of each well within 30 minutes using a microplate reader set to 450 nm.

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on log-log graph paper, with human IFN-γ concentration on the x-axis and the mean absorbance on the y-axis. Draw the best fit straight line through the standard points. To determine the test sample human IFN-γ concentration, find the mean absorbance value of the test sample 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 IFN-γ concentration. If samples were diluted, multiply by the appropriate dilution factor. Computer based curve-fitting software may be preferred. If a test sample's O.D. value falls outside the linear portion of the standard curve, the test sample should be reanalyzed at an alternate dilution as appropriate.

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Trouble Shooting

General technical hints:

- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- During incubation steps, shaking the plates may increase sensitivity.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue upon addition.
- Stop Solution should be added to the plate in the same order as the Substrate Solution.
- The color development in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that turn green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

Poor Signal

- Avoid sodium azide in wash buffers as it inhibits the enzymatic activity of HRP
- Verify that appropriate antibody pairs were used
- Inadequate reagent volumes added to wells
- Incorrect incubation times and/or temperature

Poor Standard Curve or Precision

- Improper standard handling and/or dilution
- Inadequate mixing of reagents
- Inadequate aspiration and/or washing of wells

References

1. Gray, P.W. et al. (1982) *Nature*. **295**(5849): 503-8.
2. Fleckner, J., Rasmussen, H.H. and Justesen, J. (1991) *Proc. Natl. Acad. Sci.* **88**(24): 11520-4.
3. Boehm, U. et al. (1997) *Annu. Rev. Immunol.* **15**:749-795.
4. Zamanian-Daryoush, M. et al. (2000) *Mol. Cell. Biol.* **20**:1278-1290.
5. Subramaniam, P. S. et al. (2001) *Cytokine*. **15**:175-187.
6. Meraz, M. A. et al. (1996) *Cell*. **84**:431-442.
7. Kotenko S.V. and Pestka, S. (2000) *Oncogene*. **19**(21): 2557-65.
8. Schroder, K. et al. (2004) *J. Leuk. Biol.* **75**: 163-89.
9. Mach, B. et al. (1996) *Annu. Rev. Immunol.* **14**: 301-331.
10. Anderson, S. L. et al. (1994) *J. Exp. Med.* **180**: 1565-1569.
11. Decker, T. et al. (2002) *J. Clin. Invest.* **109**: 1271-1277.
12. Huang, S. et al. (1993) *Science*. **259**: 1742-1745.
13. Collins, J. T. and Dunnick, W. A. (1993) *Int. Immunol.* **5**: 885-891.

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