Mouse IFN gamma ELISA Kit

Introduction

IFNγ, also known as IFNG, is a secreted protein that belongs to the type II interferon family. IFNγ is primarily produced by natural killer T cells and natural killer T cells as part of the innate immune response, and CD4 and CD8 cytotoxic T lymphocyte effector T cells also produce IFNγ once antigen-specific immunity is generated. It is a potent activator of macrophages, has antiproliferative effects on transformed cells, and can enhance the antiviral and antitumor effects of type I interferon.

This kit uses bispecific antibody sandwich ELISA method to detect the concentration of mouse IFNγ in the sample, with high sensitivity, strong specificity and good reproducibility. When a standard or sample is added, the mouse IFNγ will bind to the capture antibody, and then the anti-mouse IFNγ with horseradish peroxidase (HRP) is added to form a sandwich immune complex, and finally the chromogenic agent TMB solution is added, and the solid-phase captured HRP catalyzes the oxidation of TMB into a blue substance, and the absorbance value can be quantitatively detected by the microplate reader. By plotting a standard curve and comparing the absorbance values of the sample, the concentration of IFN γ in the sample can be calculated.

Components and Storage

Components \ Size	96 Tests	Storage conditions
Pre-coated plate (96-well, detachable)	1 plate	4°C
Standard	1 mL	-20°C
10× Washing Solution	50 mL	4°C
Enzyme-labeled Mouse anti-IFNγ Reaction Solution	10 mL	4°C
TMB Solution	5 mL	4°C away from light
Stop Solution	150 mL	4°C
Shipping: Blue ice	**Shelf life: 6 months**	

Protocol

1. Preparation of the sample assay: Prepare the cell culture supernatant containing Mouse IFN gamma for the test.

***Note:** Due to the complexity and unpredictable interference of natural samples such as serum, it is recommended that users explore the optimal detection conditions for natural samples with the kit.

2. Microplate plate (Pre-coated plate (96-well, detachable)) preparation: Bring all reagents back to room temperature and determine the number of wells assayed before use. Remove the unused enzyme strips from the plate frame, place them back in the foil pouch containing the desiccant pack, and reseal.

3. Standard was incubated at the same time as the test sample: 1000 μL of 8000 pg/mL of the highest concentration standard was prepared, and 500 μL was used for a two-fold dilution in a volume of 500 μL, for a total of 4 two-fold dilutions. 1× sample dilution buffer is used as a zero standard (0 pg/mL). Make sure that there is a standard curve for each assay. Do not use standard curves on other plates or on other dates. Then add 100 μL of the standard and the sample to be tested to each well. Cover or seal the plate and incubate at 37°C for 1 h. Add 200 μL of 1× Washing Buffer (1× Washing Solution) to each well and wash the plate 3 times in this manner and pat dry. Note: Improper washing may result in falsely elevated signal and poor reproducibility.

4. Enzyme-labeled antibody incubation: Add 100 μL of Enzyme-labeled Mouse anti-IFNγ Reaction Solution to each well and mix gently; Cover or seal the plate and incubate at 37°C for 1 h; Add 200 μL of 1× wash buffer per well, wash the plate 3 times in this manner, and pat dry.

5. Substrate color rendering termination and absorbance readings:

   a、Substrate chromogenicity: Add 50 μL of TMB chromogenic substrate solution (TMB Solution) to each well, mix gently, and incubate for 5 minutes at room temperature in the dark. Add 150 μL of stop solution to each well.

   b、Within 10 minutes of adding the stop solution, the absorbance of the entire plate was read at a wavelength of 450 nm.

6. Result interpretation: According to the calculation of the average absorbance of each standard and sample/the average standard optical density of the blank control >2, which is positive.

***Note:** The average standard optical density of blank control was <0.11, the positive sample was >0.22, the experiment was established.

Note

1. Product validity: The Standard product in the kit components can be stored at -20℃, and the other components can be stored at 4℃ for 6 months, and the standard product can be stored at 4℃ for 1-2 weeks.

2. Product features: Linear range: 490-8000 pg/mL, sensitivity: about 240 pg/mL.

3. The operation is strictly in accordance with the instructions, and the kits with different batch numbers of this product cannot be mixed.

4. All reagents in each group (except standard products, stored at -20℃) were stored at 4℃, balanced to room temperature before use, and could be vacuumed and stored at 4℃ or frozen at -20℃ after unpacking and

unused plates.

5. The chromogenic solution of this substrate should be stored in the dark and should not be exposed to strong light.
6. The stop solution of this product is 1 M sulfuric acid, and it must be used safely, so as not to cause the liquid to splash on the bare skin.
7. This product is for scientific research use only.

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