Instructions for Use of Human IFN-γ ELISA Detection Kit

This kit is intended for scientific use and not for diagnostic use

Cat. No. HG-IF002

Product introduction

Human IFN-γ ELISA Detection Kit uses a double-antibody sandwich method for quantitative detection of human IFN-γ protein content in serum, plasma or cell supernatant. Coat the specific anti-human IFN-γ monoclonal antibody on a microplate, add the standard , test sample, and detection antibody into the reaction wells, incubate at room temperature (18-25℃), wash, and then add the Streptavidin-HRP for incubation. After washing, add chromogenic solution TMB. The shade of the color is proportional to the target protein concentration.

Detection range: 4.69 - 300 pg/mL

Sensitivity: 0.36 pg/mL

Precision: CV% ≤ 10%, RE% ≤ ±15%

Specification

96T

Usage

The product is used for the assay of human IFN-γ protein content in serum, plasma, cell culture supernatant and other biological samples.

Kit composition

Components	Specification	Preparation
Coated Plate (CP)	8 wells x 12 strips	Ready-to-use
Human IFN-γ Standard (S)	150μL ×1 vial (3000pg/mL)	Dilute proportionally with sample diluent
Sample Diluent (SD)	15 mL × 1 vial	Ready-to-use
Detection Antibody (DA)	6 mL × 1 vial	Ready-to-use
Streptavidin-HRP (SH)	12 mL × 1 vial	Ready-to-use
Assay Buffer (AB)	12 mL × 1 vial	Ready-to-use
10x Wash Buffer (WB)	50 mL × 1 vial	Dilute with deionized water in a ratio of 1:9
TMB Substrate (TS)	12 mL × 1 vial	Ready-to-use
Stop Solution (SS)	12 mL × 1 vial	Ready-to-use
Sealer Film (SF)	5 films	Ready-to-use
Instruction For Use (IFU)	1 copy	Ready-to-use

Notes: All components are stored at 2-8℃.

Storage conditions and shelf life

The unopened kit is valid for 12 months at 2-8℃.

Materials to be self-prepared:

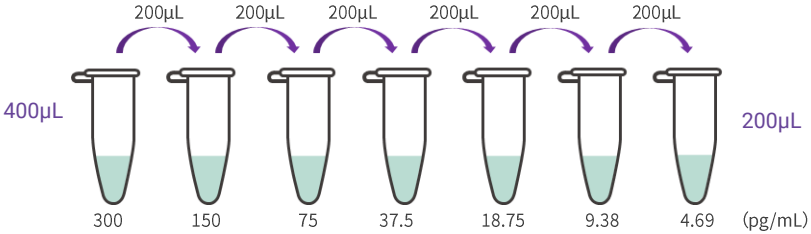
- Plate reader
- Thermostat plate shaker
- Micro pipette and tips
- Deionized water
- Unused filter paper
- Vortex shaker

Reagent configuration

1x wash solution: According to the actual amount, take an appropriate amount of 10x wash concentrate and dilute it by 10 times with deionized water.

Creation of a standard curve

Human IFN-γ Standard S 3000 pg/mL 40 µL + 360 µL SD serves as the high standard (300 pg/mL). 200 µL of SD is added to each dilution tube and 1:1 dilution series are prepared using the high standard. Thoroughly mix each tube before performing the next transfer. SD is used as a zero standard (0 pg/mL).



Operating steps

Restore all reagents and samples to room temperature (18-25℃) before testing.

1. Prepare all required reagents and working concentration standards.
2. Remove the unwanted strips, place them back into the foil pouch, and seal them again.
3. Add 50 µL detection buffer (AB) to each well.
4. Add 50 µL of standard (S) and sample. Ensure continuous spiking without interruption. The spiking process shall be completed within 15 minutes.
5. Add 50 µL of detection antibody (DA) to each well.
6. Seal the plate with a sealing film. Shake at 500 rpm and incubate at 25°C for 1 hour.

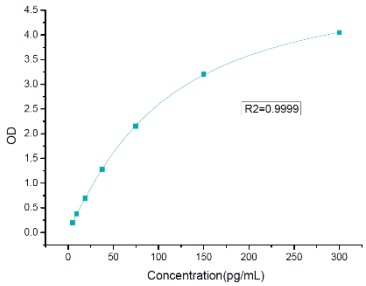
7. Discard the liquid in the wells, add 300 µL wash solution to each well to wash the plate, and wash 4 times. Each time you wash the plate, pat it dry on absorbent tissue. For ideal experimental performance, the residual liquid must be removed thoroughly.
8. Add 100 µL Streptavidin-HRP (SH).
9. Seal the plate with a new sealing film. Shake at 500 rpm and incubate at 25°C for 30 minutes.
10. Repeat step 7.
11. Add 100 µL of chromogenic solution to each well and incubate at room temperature (18-25℃) for 10-15 minutes.
12. Add 100 µL stop solution to each well.
13. Readings: Read the absorbance value at 450 nm/630 nm within 20 minutes. Take 450 nm as detection wavelength and 630 nm as reference wavelength.

Result processing

1. OD processing of the standard curve (See the following example. For example only, please refer to the actual measurement for details)

Standard concentration (pg/mL)	OD1	OD2	Mean value
300.00	4.0655	4.0291	4.0473
150.00	3.1760	3.2300	3.2030
75.00	2.1280	2.1770	2.1525
37.50	1.2630	1.2960	1.2795
18.75	0.6812	0.7103	0.6958
9.38	0.3773	0.3778	0.3776
4.69	0.1919	0.2089	0.2004
0.00	0.0218	0.0220	0.0219

2. The standard curve is obtained by 4-parameter fitting with the theoretical standard concentrations and the corresponding OD values (as shown in the figure below)



Precautions

1. When the sample is tested for the first time, it is recommended to perform dilution with at least 3 consecutive dilution factors, so as to generate at least one diluted sample within the range of the standard curve.
2. The reagents should be stored as indicated on the label, and should be equilibrated to room temperature (18-25℃) before use.
3. Before using the coated microtiter plates, please equilibrate to room temperature (18-25℃) and then open the secondary packaging. The strip plates not used in the test should be immediately placed back into the package and sealed properly, and can be stored at 4℃ for one month. Other unused reagents should be packaged or covered properly.
4. Please use disposable tips during experimental operation to avoid cross contamination.
5. Please check each individual reagent in the kit fully before use. To obtain accurate assay results, it is of special importance to mix well or shake well the reagents for dilution, loading, and reaction termination.
6. When washing residual Wash Buffer in the reaction wells, pat the plate dry adequately on clean tissue papers until watermark is no longer visible. Do not put the tissue paper into the well for liquid absorption.
7. The TMB Substrate is photosensitive, thus long-time exposure to illumination should be avoided; avoid contact with metal, otherwise, the assay results may be affected.
8. The kit is intended for single use. Please use within the shelf life.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

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