Human IFN-γ ELISA Kit

Catalog Number: HIF0111A-96

【INTENDED USE】

The kit is used for quantitative detection of Human IFN-γ in intermediate, semi-finished and finished of various biological products.

【PRINCIPLE】

This kit using double antibody sandwich enzyme-linked immunosorbent detection technology to determinate residues of Human IFN-γ in the samples. The capture antibody is coated with 96-well labeling plate and the solid phase antibody is prepared. Then first add the standard and testing samples, and second put a marker on horseradish peroxidase labeled antibody, forming a solid phase antibody- Human IFN-γ- labeled antibody sandwich, reaction after washing, then add the chromogenic reaction of the substrate. The substrate under the catalysis of HRP become blue, and under the action of stop solution into the final yellow. The absorbance (OD value) is measured at 450nm, and the content of Human IFN-γ in the sample to be tested is calculated by the standard curve.

【MATERIALS PROVIDED】

Number	Component	Size	Storage condition
Box1	Microtiter Plate	96T	2-8℃
	Sample Dilution	30 mL×1 vial	2-8℃
	HRP-Conjugate Antibody(10×)	1.5 mL×1 tube	2-8℃
	HRP-Conjugate Antibody Dilution	15 mL×1 vial	2-8℃
	Wash Buffer (20×)	30 mL×1 vial	2-8℃
	Developing solution A	8 mL×1 vial	2-8℃ (keep in dark)
	Developing solution B	8 mL×1 vial	2-8℃ (keep in dark)
	Stop Solution	15 mL×1 vial	2-8℃
	Microplate Sealers	3	-
Box2	Standard (200 ng/mL)	300 μL× 1 tube	-20℃

【STORAGE】

The standard is stored at -20℃, and the remaining components are stored at 2-8℃, and the validity period is 12 months. Kits that have not been used after opening should be kept under the specified storage conditions.

The production date and expiration date can be found on the kit label.

【REAGENTS/EQUIPMENTS REQUIRED BUT NOT SUPPLIED】

1. Enzyme-labeled instrument, constant temperature oscillator (or constant temperature incubator), auto microplate-washer（ Not recommended for cross-use with other projects Or Direct hand wash), whirlpool oscillator, timer
2. High-precision pipette and disposable suction head (0.5-10μL, 10-100μL, 30-300μL, 100-1000μL)
3. Sterile deionized water without heat source
4. Absorbent paper, EP tube, disposable gloves

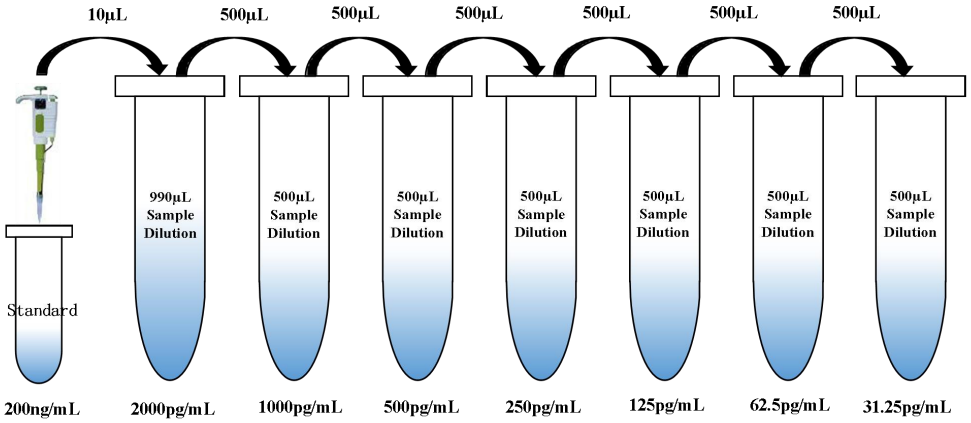
【REAGENT PREPARATION】

- Wash Buffer(1×):Take 1 part of Wash Buffer (20×) and add 19 parts of deionized water to prepare a working concentration wash buffer (1×). If there is crystallization in the wash buffer (20×), it should be gently shaken at room temperature or 37℃ in a water bath, and then diluted after the crystallization is completely dissolved. Unused wash buffer (20×) should be stored at 2-8℃.
- Standard preparation: Standards are diluted to 2000 pg/mL with dilution and then prepared by 2 times the dilution method.（When the standard product is diluted in gradient, it is necessary to use the pipette to blow and suck for 5 to 10 times, and fully mix, and avoid bubbles when the pipette blows and sucks. Do not use vortex oscillator to mix standard products when diluting and adding samples.）
- Preparation of HRP-Conjugate Antibody(1×): the HRP-Conjugate antibody (10×) was diluted 10 times with HRP-Conjugate Antibody Dilution to become HRP-Conjugate antibody (1×). For example, 100 μL HRP-Conjugate Antibody（10×） is added to 900 μL diluent.
- Substrate Solution: Mix the developing solution A and developing solution B with equal volume before 10 minutes using. Avoid light to ensure that the substrate solution is not contaminated. If the substrate solution after mixing has turned blue, do not use it.

【ASSAY PROCEDURE】

Bring all reagents and samples to room temperature before use.

1. Bring the test slats from the aluminum foil bag that has been balanced to room temperature, put the remaining slats back into the aluminum foil bag, seal them, and store them in 2-8℃ .
2. Preparation of the standard curve: dilute the Standard with Sample dilution to 2000pg/mL, then prepared by Multiple Dilution method.



3. Temperature breeding of samples: set up standard wells, blank wells and sample wells. 100 μL of standard substances of different concentrations were added to the standard wells in order, and 100 μL of Dilution to the blank wells, and 100 μL of samples to be tested were added to the sample wells, and then incubate at 37℃ for 1h.

4. Washing board: discard the liquid in the well and wash the board with wash buffer (1×) for 3 times (250 μL /well). Pat dry residual liquid in sample well. (Before discarding the liquid in the well each time, if washing plate by hand, the liquid can be left for 1min after adding and slightly shaken; If plate washing machine is used, 5s can be slightly shaken after adding wash buffer).

5. HRP-conjugate antibody incubate: Add 100 μL of HRP-conjugate antibody(1×) each well, and then incubate at 37℃ for 1h.

6. Washing board: discard the liquid in the well and wash the board with wash buffer (1×) for 3 times (250 μL /well). Pat dry residual liquid in sample well.

7. Color development: the pre-prepared substrate solution was added to the enzyme label plate, mixed well (100 μL/well), and incubated in 37℃ for 15 min.

8. Termination: add stop solution (100 μL /well).

9. Recording: The OD value at 450 nm/630 nm should be measured by placing the plate in the microcoder, and the reading should be completed within 10 min.

【CALCULATION OF RESULTS】

1. Calculation of absorbance value: The calibrated value of light absorbance of each standard or sample is: ($OD_{450\ nm}$-$OD_{630\ nm}$)- absorbance valueof the blank control well ($OD_{450\ nm}$-$OD_{630\ nm}$).
2. Plot the standard curve with concentration of the standard as the horizontal coordinate (X) and the calibrated value of light absorption of the standard as the vertical coordinate (Y), and a four-parameter Logistic mathematical model is recommended to fit the equation:Y=（（A-D）/（1+（X/C）^B））+D.Substitute OD value of the sample ($OD_{450\ nm}$-$OD_{630\ nm}$) -

absorbance value of the blank control well($OD_{450\ nm}$-$OD_{630\ nm}$)) into the formula to calculate the content of Human IFN-γ in the sample, and note the dilution factor.

3. If the OD value of the sample to be tested exceeds the highest OD value of the standard curve, the sample shall be redetermined after dilution.

【PRECAUTIONS】

1. This kit is only used for in study, not for clinical diagnosis.
2. This kit should not be used beyond the expiration date.
3. All reagents in the kit must be restored to room temperature (20-25℃) before use.
4. The best detection effect can be guaranteed only by strictly following the operation method of the instructions and using all the reagents matched with the kit.
5. Each test should prepare the corresponding standard curve. DO NOT USE the standard curves from different kits or different batches.
6. Pay attention to the timely replacement of sample adding tank and pipette tips between different samples and steps to avoid cross contamination.
7. The final test results are closely related to the effectiveness of the reagent the operator's method of operation and the test environment.
8. The company is only responsible for the kit itself and is not responsible for the sample consumption caused by the use of the kit. Users are requested to take full account of the possible usage of the sample and reserve sufficient samples before using the kit.

【SAFETY INSTRUCTIONS】

1. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.
2. Personal protective equipment such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.

【PROBLEM ANALYSIS】

If there is any problem with the experimental results, please take photos of the color rendering results in time, and properly preserve the unused slats and reagents. Then contact technical support to find out the reason.

Problem Description	Possible Reasons	Solution
Gradient difference of standard curve	Dilution error	The standard curve is not diluted according to the dilution factor
	The liquids are aspirated or added inaccurately	Check the pipette and tips
	The ELISA plates are not washed completely	Guarantee the plate washing times, and the amount used for washing liquid per well

The color development is weak or colorless	The incubation time is too short	Guarantee sufficient incubation time
	The experiment temperature is not correct	Use the recommended incubation temperature
	The reagent volume is insufficient or miss to add the reagent	Check the liquid aspiration and adding process, and guarantee all the reagents are added sufficiently in sequence
	The developing solution are prepared wrongly	The developing solutions A and B are mixed in same volume and protected from light, which are prepared 10 min before color development
The OD value are of low reading	The sample is not treated	The samples are tested after centrifugation by heating
	The plate reader are set incorrectly	Check the wavelength and optical filter device on the plate reader
		The plate reader shall be opened in advance before reading for preheating
The OD value are of high reading	The sample is not diluted	It is recommended to validate the dilution factor of the sample
The coefficient of variation (CV value) is large	The liquid adding is incorrect	Check the liquid adding condition
	There are pollution at the bottom of the ELISA plate	Check the bottom of the ELISA plate for residual liquid or handprint
	There are foreign matters or bubbles in the plate well	Confirm that there are no foreign matters in the plate well before adding samples, and confirm that there are no bubbles after adding samples
	The plate is not sealed or sealed incompletely during incubation	Seal the plate with a plate sealer
The background value is high	The ELISA plates are not washed completely	Wash the plate according to the method recommended in the instruction for use
		If automatic plate washer is used, please check all the liquid adding port and waste liquid outlet for blocking
		If the plate is washed by hands, the plate washing times can be added properly

		Insufficient washing or missing to wash may lead to high background
	Incubation time and temperature	Operate in strict accordance with requirements in the instruction for use
	Consumption pollution	The consumables used (such as tubes, tips etc.) are not clean
	The washing solution is polluted	Prepare fresh washing solution
	The developing solution is polluted	The color developing solution itself does not have colors, please ensure that the color developing solution is not polluted by metal ion or oxidizing agent before use, and it is protected from light.
The sensitivity is low	The kit is stored improperly	Store the related reagents in accordance with the requirements in the instruction for use

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