Human IFN-γ Precoated ELISA kit

(Cat#： 1110002)

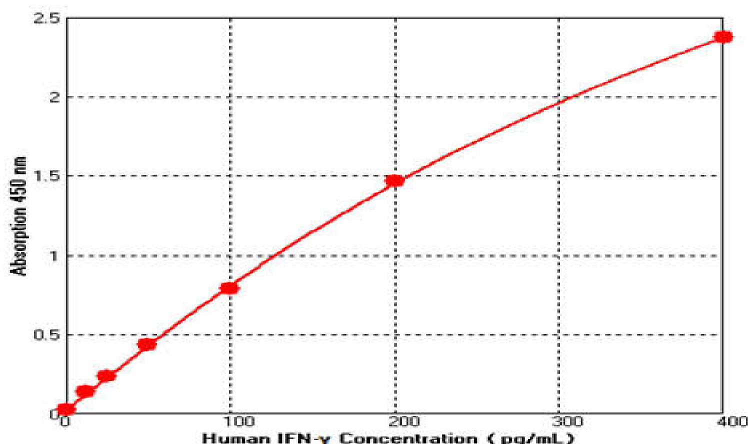
Product Description:

The Human IFN-γ ELISA kit is to be used for the in-vitro quantitative determination of IFN-γ in human serum, plasma, buffered solutions or cell culture medium. The assay will recognize both natural and recombinant human IFN-γ. **This kit has been configured for research use only and is not to be used in diagnostic or therapeutic procedures.** Read the manual and check the reagents in the kit before use If there are any questions, please contact us by sending E-mail to: rd@dakewe.com.

Standard Curve Range: 12.5-400 pg/mL

Sensitivity: 5 pg/mL

Reproducibility: Intra-assay CV: ≤10%; Inter-assay CV: ≤15%



Note: Typical IFN-γ standard curve ranging from 12.5 to 400 pg/mL

Reagents Provided and Reconstitution:

Reagents	Quantity	Reconstitution
Cytokine standard	2 vials	Lyophilized, reconstitute with the volume of reagent indicated on the vial
Biotinylated antibody	2 vials	Dilute 50 times in Diluent buffer（1×）
Streptavidin-HRP	2 vials	Dilute 100 times in Diluent buffer（1×）
Diluent buffer（1×）	3 vials	Ready-to-use
Washing buffer（50×）	1 vial	Dilute 50 times in distilled Water
TMB	1 vial	Ready-to-use
Stop solution	1 vial	Ready-to-use
Precoated ELISA plates	8×12	Ready-to-use
Plastic cover	4 sheets	Ready-to-use

Material Required but not Provided:

1. 96-well ELISA plate (microplate spectrophotometer).
2. Pipettes and pipette tips: 10 μL, 50 μL, 100 μL, 200 μL and 1000 μL.
3. Distilled water, fresh absorbent paper.
4. Vortex mixer and magnetic stirrer.

Protocol Notes/ Lab. Quality Control:

1. When not in use, kit components should be stored refrigerated or frozen as indicated on vials or bottles labels. All reagents should be warmed to room temperature before use. Lyophilized standards should be discarded after use.
2. Centrifuge Streptavidin-HRP and Biotinylated antibody immediately before use.
3. Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration. Cover or cap all reagents when not in use.
4. Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross-contamination.
5. Thoroughly mix the reagents and samples before use by agitation or swirling.
6. When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
7. Use a clean plastic container to prepare the washing solution.
8. All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by

tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.

9. The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent color development. Warning TMB is toxic avoid direct contact with hands. Dispose of TMB when it turns blue. Please read absorbance within 10 minutes after adding the stop solution.
10. Respect incubation times and temperature described in the assay procedure.
11. Do not use reagents beyond the expiration date of the kit. Do not mix or interchange reagents between different lots.
12. Do not extrapolate the standard curve range. The dose-response is non-linear out of the range and good accuracy is difficult to obtain.
13. Specificity: No cross-reactivity to other human cytokines.

Preparation of Reagents:

1. **Cytokine standard：** First reconstitute the freeze-dried material with the volume of reagent indicated on the vial. Stand for 5 minutes and mix gently to ensure complete solubilization. Dilute the standard to recommending concentration with Diluent buffer（1×）, **Serial dilution of standard to 400 pg/mL, 200 pg/mL, 100 pg/mL, 50 pg/mL, 25 pg/mL, 12.5 pg/mL is recommended.**
2. **Biotinylated antibody：** Dilute 50 times with Diluent buffer（1×）in a clean glass vial as needed according to the following table:

Number of Wells used	Biotinylated Antibody (μL)	Diluent buffer（1×）(μL)
16	17	850
24	25	1250
32	34	1700
48	50	2500
96	100	5000

3. **Streptavidin-HRP ：** Dilute 100 times with Diluent buffer（1×）in a clean glass vial as needed according to the following table:

Number of Wells	Streptavidin-HRP (μL)	Diluent buffer（1×）(mL)
16	17	1.7
24	25	2.5
32	34	3.4
48	50	5
96	100	10

4. **Washing buffer(50×)：** Dilute 50 times in distilled water.

Assay Method:

1. Before use, mix all reagents thoroughly without making foam.
2. Determine the number of microwell strips required to test the desired number of samples, plus appropriate number of wells needed for running blanks and standards. Each sample, standard and blank should be assayed in duplicate. Remove sufficient microwell strips from the pouch.
3. Add 100 μL of diluted Cytokine standard to standard wells, 100 μL of sample to sample wells, 100 μL of standard diluent to the blank wells.
4. Add 50 μL of diluted Biotinylated antibody to all wells. Cover with a plate cover and incubate 2 hours at room temperature (18°C - 25°C).
5. Remove the cover and wash the plate as follows: Aspirate the wells to remove liquid and wash the plate 3 times using 300 μL of wash buffer per well. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess wash buffer. Use the microwell strips immediately after washing.
6. Add 100 μL of diluted streptavidin-HRP to all wells. Cover and incubate 20 minutes at room temperature.
7. Remove the cover and empty wells. Wash microwell strips according to step 5. Proceed immediately to the next step.
8. Pipette 100 μL of TMB substrate solution into all wells, including the blank wells and incubate in the dark for 10-30

minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.

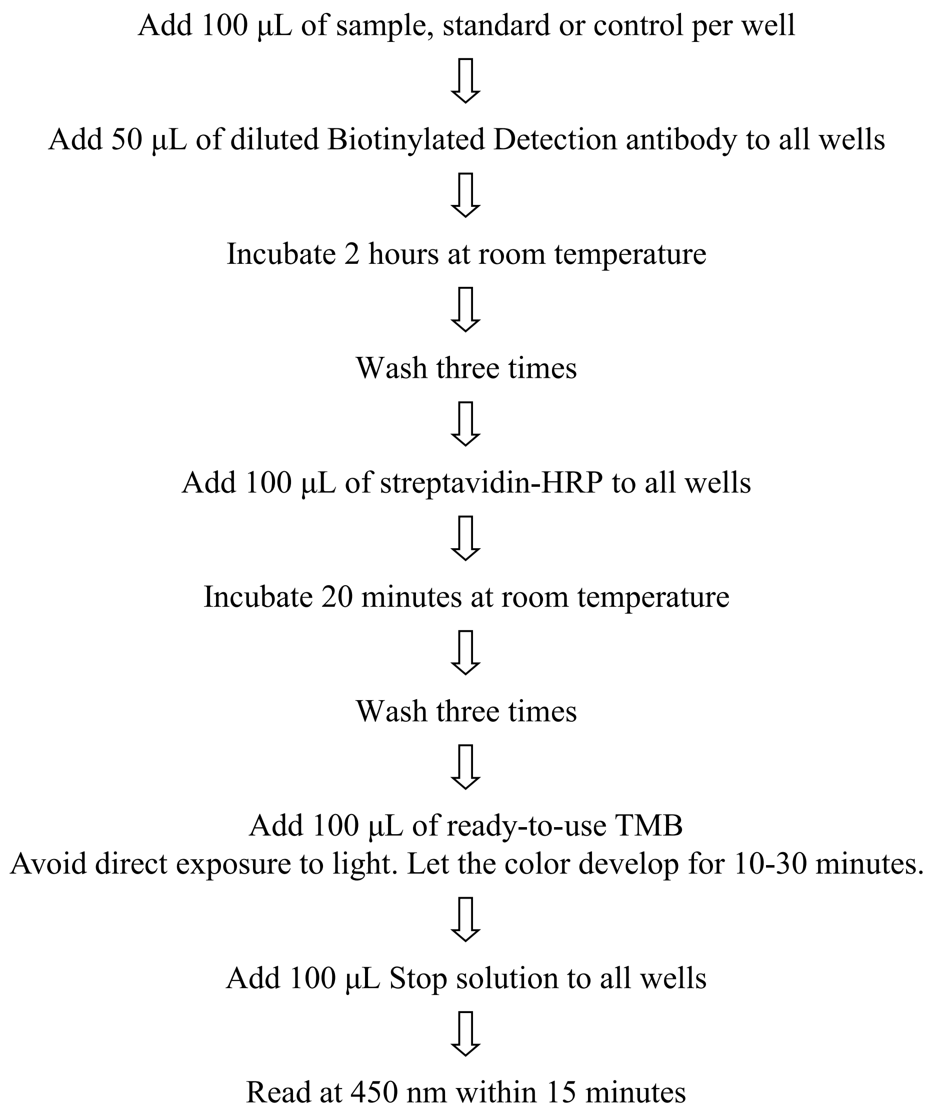
9. The enzyme-substrate reaction is stopped by quickly pipetting 100 μL of Stop solution into each well.

10. Measure the optical density in an ELISA reader at 450 nm (optionally 610-650 nm as the reference wave length) within 15 minutes. Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of the samples and the standards.

Data Analysis:

a) Generate a standard curve by reducing the data using computer software capable of generation a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
b) If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Assay Procedure Summary:



Appendix:

ELISA Troubleshooting Guide		
Problem	Possibility	Solution
1. Very weak signal	(1) Improper and inefficient washing	(1) correct Washing
	(2) Reagents have expired	(2) Do not use the expired reagents
	(3) Incorrect dilutions, conjugate concentration was too low	(3) Repeat test using correct dilutions, check with the recommendations of the antibody manufacturer
	(4) Inefficient incubation time	(4) Repeat ELISA, follow the protocol carefully for each step' s incubation time
	(5) Incorrect storage of reagents	(5) Store reagents in the correct temperature, avoid freeze and thaw, avoid using the "frost free" freezer
	(6) Wrong filter in ELISA reader was used	(6) Use the correct wavelength setting
2. Poor standard curve and duplicates	(1) Improper and inefficient washing	(1) correct Washing
	(2) Poor mixing of samples	(2) Mix samples and reagents gently and warm to room temperature
	(3) Plates not clean	(3) Plates should be wiped on bottom before measuring absorbance
	(4) Wrong filter in ELISA reader was used	(4) Use the correct wavelength setting
	(5) Mix or cross-use reagents between different lots	(5) Use reagents from the same kit
	(6) Cross-contamination between wells	(6) Use a clean disposable plastic pipette tip for each reagent, standard or sample addition in order to avoid cross-contamination
	(7) Samples and reagents were absorbed to the uncoated district	(7) Adding the liquid by gently lowering an aspiration tip into the center of each well
	(8) Inconsistent incubation, washing and color development time	(8) Use a timer
3. No signal	(1) Enzyme inhibitor present in your system e.g., sodium azide in the washing buffer and diluent buffer inhibits peroxidase activity	(1) Repeat ELISA, make sure your system contains no enzyme inhibitor
	(2) Wrong reagents were used or some steps have been omitted	(2) Repeat ELISA, follow the protocol carefully and use the correct reagents
	(3) Reagents have expired	(3) Do not use the expired reagents
4. High background	(1) Improper and inefficient washing	(1) correct Washing
	(2) Contaminated substrate	(2) Substrate should be colorless
	(3) Reagents have expired	(3) Do not use the expired reagents
	(4) Incorrect dilutions, e.g., conjugate concentration was too high	(4) Repeat test using correct dilutions, check with the recommendations of the antibody manufacture
	(5) Distilled water contaminated by HRP et al.	(5) Use fresh distilled water
	(6) Cross contamination with other positive samples	(6) Repeat ELISA, be careful when washing and pipetting
	(7) Incubator temperature over 37℃ or reaction time is too long	(7) Calibrate the incubator temperature; shorten the color development time

DAKEWE Bio-engineering Co., LTD.(SHEN ZHEN)

702 、 703 ， Building No.1 ， Shenzhen Biomedicine Innovations Industrial Park, No.14 Jinhui Road, Pingshan District, Shenzhen 518118 Tel: 0755-86235300 Fax:0755-86235303 Web:www.dakewe.com E-mail:RD@dakewe.com （180701）