Version 22-04/24

User's Manual

Human IFN Gamma ELISA Kit

REF **DEIA-JY2137**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Enzyme immunoassay for determination of IFN Gamma.

Principles of Testing

The microtiter plate provided in this kit has been pre-coated with an antibody specific to Interferon Gamma (IFNγ). Standards or samples are added to the appropriate microtiter plate wells along with a biotin-conjugated antibody specific to Interferon Gamma (IFNγ). Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After adding the TMB substrate solution, only those wells that contain Interferon Gamma (IFNγ), biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by adding sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm±10nm. The concentration of Interferon Gamma (IFNγ) in the samples is determined by comparing the OD of the samples to the standard curve.

Reagents And Materials Provided

Reagents	Quantity		Storage Condition
	48T	96T	
Pre-Coated Microplate	6 strips x 8 wells	12 strips x 8 wells	-20°C (6 months)
Standard (Lyophilized)	1 vial	2 vials	-20°C (6 months)
Biotinylated Antibody (100×)	60 µL	120 µL	-20°C (6 months)
Streptavidin-HRP (100×)	60 µL	120 µL	-20°C (6 months)
Standard/Sample Diluent Buffer	10 mL	20 mL	4°C
Biotinylated Antibody Diluent	6 mL	12 mL	4°C
HRP Diluent	6 mL	12 mL	4°C
Wash Buffer (25×)	10 mL	20 mL	4°C
TMB Substrate Solution	6 mL	10 mL	4°C (store in dark)
Stop Reagent	3 mL	6 mL	4°C
Plate Covers	1 piece	2 pieces	4°C

1. Please store the kit at 4°C if used up in 1 week. If used for more than 1 week, store the Pre-Coated Microplate, Standard, Biotinylated-Conjugate and Streptavidin-HRP at -20°C and all other reagents at 4°C according to the temperature indicated on the label. Avoid repeated freeze-thaw cycles.
2. Do not use the kit beyond the expiration date.
3. After opening the package, please check that all components are complete.
4. The cap must be tightened to prevent evaporation and microbial contamination. The reagents volume is slightly more than the volume marked on labels, please use accurate measuring equipment and do not pour directly into the vial.

Note: All kit components have been formulated and quality control tested to function successfully. Do not mix or substitute reagents or materials from other kits, detection effect of the kit will not be guaranteed if utilized separately or substituted.

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 ± 10 nm.
2. High-speed centrifuge.
3. Electro-heating standing-temperature cultivator.
4. Absorbent paper.
5. Double distilled water or deionized water.
6. Single or multi-channel pipettes with high precision and disposable tips.
7. Precision pipettes to deliver 2 µL to 1 mL volumes.

Storage

2-8°C

Specimen Collection And Preparation

Serum - Samples should be collected into a serum separator tube. After clotting for 2 hours at room temperature or overnight at 4°C, and then centrifuging at 1000 ×g for 20 minutes. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 1000 ×g and 2-8°C for 15 minutes within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type.

1. Rinse the tissues in pre-cooled PBS to completely remove excess blood, and weigh them before homogenization.
2. Mince the tissues to small pieces and homogenize them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular location of the target protein) (PBS can be used as the lysis buffer of most tissues) (w:v = 1:9, e.g. 900 µL lysis buffer is added in 100 mg tissue sample) with a glass homogenizer on ice (micro tissue grinders, too).
3. Ultrasound the obtained suspension with an ultrasonic cell disrupter until the solution is clear.
4. Then, centrifuge the homogenates for 5 minutes at 10000 ×g and collect the supernatant and assay immediately or store in aliquots at ≤ -20°C.

   *Note: Tissue homogenates are recommended to be tested for protein concentration at the same time to obtain a more accurate concentration of the test substance per mg of protein.

Cell lysates - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by pre-cooled PBS gently, and then be detached with trypsin, and collect them by centrifugation at 1000 ×g for 5 minutes (suspension cells can be collected by centrifugation

directly).

2. Wash cells 3 times in pre-cooled PBS.
3. Then, resuspend the cells in fresh lysis buffer with concentration of 10^7 cells/mL. If it is necessary, the cells could be subjected to ultrasonication until the solution is clear.
4. Centrifuge at 1500 ×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or store in aliquots at ≤ -20°C.

Urine - Collect the first urine of the day (mid-stream) and discharge it directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Saliva - Collect saliva using a collection device or equivalent. Centrifuge samples at 1000 ×g at 2-8°C for 15 minutes. Remove particulates and assay immediately or store samples in aliquot at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Cell culture supernatants and other biological fluids - Centrifuge samples at 1000 ×g for 20 minutes. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Feces - Dry feces were collected as much as possible, weighing more than 50 mg. The feces wer ewashed three times with PBS (w:v = 1:9, e.g. 900 µL lysis buffer is added in 100 mg feces), sonicated (or mashed)and centrifuged at 5000 ×g for 10 minutes, where the supernatant was collected for testing.

Cerebrospinal fluid (CSF) - Remove particulates by centrifugation and assay immediately or aliquotandstore samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Notes

1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bio-activity and contamination. Avoid repeated freeze-thaw cycles.
2. Sample hemolysis will influence the result, so it should not be used.
3. When performing the assay, bring samples to room temperature.

   If the concentration of the test material in your sample is higher than that of the Standard product, please make the appropriate multiple dilutions according to the actual situation (it is recommended to do preliminary experiment to determine the dilution ratio.

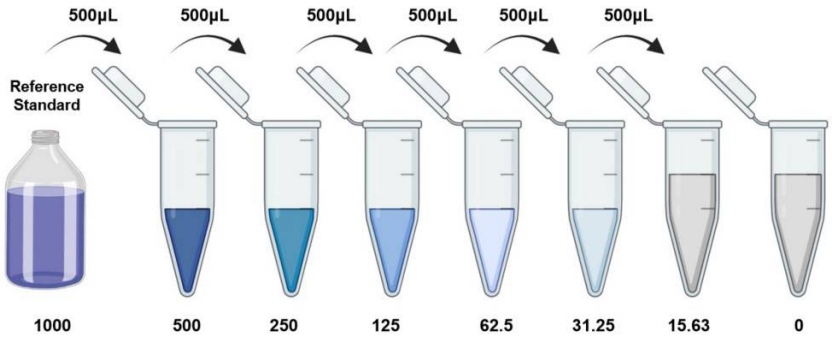
Samples Preparation

1. Equilibrate all materials and prepared reagents to room temperature prior to use. Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.
2. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
3. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

Reagent Preparation

1. Bring all kit components and samples to room temperature (18-25°C) before use.

2. If the kit will not be used up in 1 time, please only take out strips and reagents for present experiment, and save the remaining strips and reagents as specified.
3. Dilute the 25× Wash Buffer into 1× Wash Buffer with double distilled water.
4. **Standard Working Solution** - Centrifuge the Standard at 1000 ×g for 1 minute. Reconstitute the Standard with 1.0 mL of Standard Diluent Buffer, kept for about 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 100 ng/mL. Please prepare 7 tubes containing 0.5 mL Standard Diluent Buffer and use the Diluted Standard to produce a double dilution series according to the picture shown below. To mix each tube thoroughly before the next transfer, pipette the solution up and down several times. Set up 7 points of Diluted Standard such as 1000 ng/mL, 500 ng/mL, 250 ng/mL, 125 ng/mL, 62.5 ng/mL, 31.25 ng/mL, 15.63 ng/mL, and the last EP tubes with Standard Diluent is the **Blank** as 0 ng/mL. In order to guarantee the experimental results validity, please use the new Standard Solution for each experiment. When diluting the Standard from high concentration to low concentration, replace the pipette tip for each dilution. **Note:** the last tube is regarded as the **Blank** and **do not** pipette solution into it from the former tube.



5. **1× Biotinylated-Antibody and 1× Streptavidin-HRP Working Solution** - Briefly spin or centrifuge the stock Biotinylated-Antibody and Streptavidin-HRP before use. Dilute them to the working concentration 100-fold with Biotinylated-Antibody Diluent and HRP Diluent, respectively. For example, 10 µL of Streptavidin-HRP with 990 µL of HRP Diluent.
6. **TMB Substrate Solution** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Notes

1. After receiving the kit, please store the reagents according to the instructions. The plates can be disassembled into single strips. Please use them in batches as needed. It is recommended that the remaining reagents be used within 1 month after the first test.
2. The test tubes, pipette tips, and reagents used in the experiment are all disposable and strictly prohibited from being reused; otherwise, the experiment's results will be affected. Kit reagents of different batches cannot be mixed (except for TMB, Washing Buffer, and Stop Reagent).
3. Lyophilized Standards, Biotinylated-Conjugate, and Streptavidin-HRP are small in volume and may scatter in various parts of the tube during transportation. Please centrifuge them at 1000xg for 1 minute before use. Then, carefully pipette 4-5 times to mix the solution. Please configure the Standard, 1× Biotinylated-Conjugate, and 1× Streptavidin-HRP working solution according to the required amount and use the corresponding Dilution Solution. They cannot be mixed and used.
4. Bring all reagents to room temperature (18-25°C) before use. If crystals form in the concentrate (25×), it is a normal phenomenon. Heat it to room temperature (the heating temperature should not exceed 40°C) and

gently mix it until the crystals completely dissolve.

5. Prepare to dissolve the Standard within 15 minutes before the test. This Standard Working Solution can only be used once. If the dissolved Standard is not used up, please discard it. The sample addition needs to be rapid. Each sample addition should preferably be controlled within 10 minutes. To ensure experimental accuracy, it is recommended to test duplicate wells. In addition, when pipetting reagents, keep a consistent order of additions from one well to another, which will ensure the same incubation time for all wells.
6. During the washing process, pat the residual washing liquid in the reaction well dry on absorbent paper. Do not put the paper directly into the reaction well to absorb water. Before reading, remove the residual liquid and fingerprints at the bottom, making sure not to affect the microplate reader reading.
7. TMB Substrate Solution is light-sensitive, so avoid prolonged exposure to light. Dispense the TMB Substrate Solution within 15 minutes following the washing of the microtiter plate. Also, avoid contact between TMB Substrate Solution and metal to prevent color development. TMB is contaminated if it turns blue before use and should be discarded. TMB is toxic, so avoid direct contact with hands.
8. Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.

Assay Procedure

1. Determine which wells to use for the Diluted Standard, Blank, and Sample. Prepare 7 wells for the Standard and 1 well for the Blank. Add 100 µL of Standard Working Solution (see Reagent Preparation), or 100 µL of samples to the appropriate wells. Cover with the Plate Cover and incubate at 37°C for 80 minutes. Note: Please add solutions to the bottom of the micro ELISA plate well, avoiding touching the inside wall and causing foaming as much as possible.
2. Pour out the liquid from each well. Aspirate the solution and wash each well with 200 µL of 1×Wash Solution, allowing it to sit for 1-2 minutes. Completely remove any remaining liquid from all wells by snapping the plate onto absorbent paper. Wash the plate 3 times, removing any remaining Wash Buffer by decanting or aspirating. After the last wash, invert the plate and blot it against absorbent paper.

Note: (a) When adding Washing Solution, the pipette tip should not touch the wall of the wells to avoid contamination. (b) Pay attention to pouring the washing liquid directly to ensure that the washing liquid does not contaminate other wells.

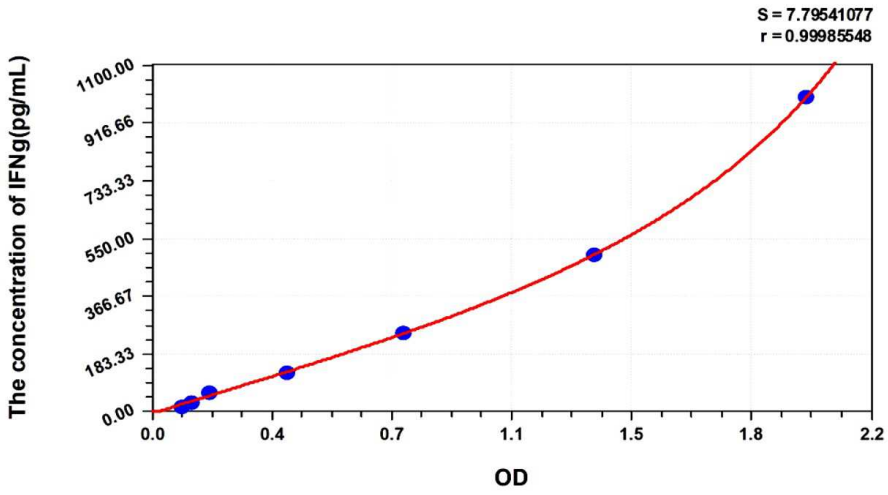
3. Add 100 µL of Biotinylated Antibody Working Solution to each well. Cover the wells with the Plate Cover and incubate at 37°C for 50 minutes.
4. Repeat the aspiration and wash process for a total of 3 times as conducted in step 2.
5. Add 100 µL of Streptavidin-HRP Working Solution to each well. Cover the wells with the plate sealer and incubate at 37°C for 50 minutes.
6. Repeat the aspiration and wash process for a total of 5 times as conducted in step 2.
7. Add 90 µL of TMB Substrate Solution to each well. Cover with a new Plate Cover and incubate for 20 minutes at 37°C (Do not exceed 30 minutes) in the dark. The liquid will turn blue with the addition of TMB Substrate Solution. Preheat the Microplate Reader for about 15 minutes before OD measurement.
8. Add 50 µL of Stop Reagent to each well. The liquid will turn yellow with the addition of Stop Reagent. Mix the liquid by tapping the side of the plate. If color change is not uniform, gently tap the plate to ensure thorough mixing. The order of insertion of the Stop Reagent should be the same as that of the TMB Substrate Solution.

9. Wipe off any water drops and fingerprints on the bottom of the plate and confirm that there are no bubbles on the surface of the liquid. Then, run the microplate reader and immediately conduct measurement at 450 nm.

Typical Standard Curve

Average the duplicate readings for each Standard, Control, and Sample, subtract the average zero Standard optical density. Construct a Standard curve with the Human IFNγ concentration on the y-axis and the absorbance on the x-axis. Draw a best-fit curve through the points on the graph. If samples have been diluted, multiply the concentration read from the Standard curve by the dilution factor. Plot the curve using a software such as Curve Expert.

Concentration (pg/mL)	OD	Corrected OD
1000	2.128	1.998
500	1.482	1.352
250	0.896	0.766
125	0.543	0.413
62.5	0.306	0.176
31.25	0.251	0.121
15.63	0.222	0.092
0	0.13	0.000



Precision

Intra-assay Precision (Precision within an assay):CV% < 8%

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays):CV% < 10%

Three samples of known concentration were tested in forty separate assays to assess inter-assay precision.

Detection Range

15.63 - 1000 pg/mL

Sensitivity

5.9 pg/mL

Specificity

This assay has high sensitivity and excellent specificity for detection of Human IFNγ. Nosignificant cross-reactivity or interference between Human IFNγ and analogues was observed.

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of IFNγ and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8	1:16
Serum (*n* = 5)	86-93%	91-103%	87-98%	95-101%
EDTA plasma (*n* = 5)	89-99%	83-95%	98-104%	96-106%
Heparin plasma (*n* = 5)	85-100%	84-98%	95-101%	88-102%

Recovery

Matrices listed below were spiked with certain level of recombinant IFNγ and the recovery rates were calculated by comparing the measured value to the expected amount of IFNγ in samples.

Precautions

1. This kit is only used for lab research and development and should not be used for human or animals.
2. Reagents should be regarded as hazardous substances and should be handled carefully and correctly.
3. Gloves, lab coats, and goggles should always be worn to avoid skin and eyes coming into contact with Stop Reagent and TMB. In case of contact, wash thoroughly with water.