Add&Read Human IFN gamma Quantitative Detection Kit

DD2706

Vazyme

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Vazyme

Instruction for Use

Version 24.1

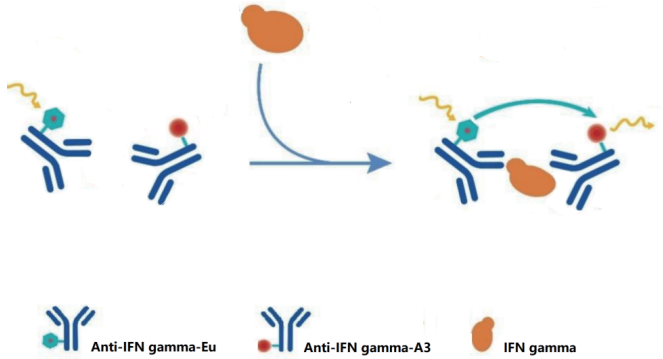
目录 Contents

For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

Interferon-gamma (IFN gamma) is a pro-inflammatory cytokine primarily produced by T cells and natural killer (NK) cells, playing a crucial role in antiviral defense mechanisms within the body. IFN-gamma assembles into soluble dimers intracellularly and is secreted into the extracellular space. It is involved in antiviral defense, activation of macrophages, enhancement of neutrophil and monocyte function, and upregulation of MHC-I and MHC-II expression in cells.

This reagent kit utilizes a sandwich method to detect IFN gamma levels. The kit contains IFN gamma Standard and two monoclonal antibodies specific to IFN gamma, one coupled with Eu (donor, Anti-IFN gamma-Eu) and the other coupled with A3 (acceptor, Anti-IFN gamma-A3). When both antibodies bind to IFN gamma simultaneously, the proximity of Anti-IFN gamma-Eu and Anti-IFN gamma-A3 allows for fluorescence resonance energy transfer (FRET) to occur. Use 320/340 nm excitation light to excite the fluorescent donor, which emits 620 nm light. The 620 nm light excites the fluorescent receptor, which emits 665 nm light. The IFN gamma concentration in the sample is proportional to the FRET signal (ratio of fluorescence intensity at 665 nm to 620 nm).



02/Product Components

Components	DD2706-01(96 tests)	DD2706-02(500 tests)	DD2706-03(10,000 tests)
IFN gamma Standard (Lyophilized)	1 Vial	2 Vial	4 Vial
Anti-IFN gamma-Eu (20 ×)	12 μl	50 μl	1 ml
Anti-IFN gamma-A3 (20 ×)	12 μl	50 μl	1 ml
Detection Buffer (ready-to-use)	500 μl	3 ml	50 ml
Diluent Buffer (ready-to-use)	2 × 1 ml	10 ml	100 ml
Manual	1 volume	1 volume	1 volume

03/Storage Conditions and Validity Period

Store at -30℃ ~ -15℃ and transport at ≤0℃. The reagent kit should protect from light. The validity period of the kit is 12 months.

04/Scope of Application

Cellular supernatant

05/Self-provided Materials

96/384-well low volume white plate

Microplate reader (with HTRF/TR-FRET module)

06/Precautions

1. Anti-IFN gamma-Eu (20 ×) and Anti-IFN gamma-A3 (20 ×) are recommended to be aliquoted and stored at -30℃ ~ -15℃, avoiding repeated freeze-thaw cycles. The aliquot volume is suggested to be more than 10 µl.
2. The reconstituted IFN gamma Std is recommended to be stored at -85℃ ~ -65℃, avoiding repeated freeze-thaw cycles.
3. If slight precipitation occurs in the Detection Buffer and Diluent Buffer, it is considered normal. The precipitation could be gently vortexed at room temperature or a 37℃ water bath to dissolve it before normal use. Thawed Detection Buffer and Diluent Buffer can be stored at 2℃ ~ 8℃.
4. To check for potential interference effects in your detection buffer during the first use of this reagent kit, we recommend preparing calibration curves in parallel using your own culture medium and diluent.
5. Avoid bubble formation when adding samples.

07/Experiment Process

07-1/Reagent Preparation

1. Preparation of Anti-IFN gamma-Eu and Anti-IFN gamma-A3 working solutions (stock solution is 20 ×)

   For a reaction volume of 20 µl in a 96/384-well low volume white plate, it is recommended to add 2 µl of Anti-IFN gamma-Eu and 2 µl of Anti-IFN gamma-A3 working solutions to each 20 µl system. Before preparing, calculate the required volumes of Anti-IFN gamma-Eu (20 ×) and Anti-IFN gamma-A3 (20×) as follows: V = (number of sample wells × 2/20) µl.

   ▲ When calculating the number of sampling wells, the pipetting loss should be taken into account. Generally recommended: Number of sampling wells = Actual number of detection wells × 110%.

   Preparation of Anti-IFN gamma-Eu working solution:

   ● Remove Anti-IFN gamma-Eu (20 ×) from the refrigerator and allow it to thaw at room temperature until completely dissolved. Mix thoroughly before use.

   ● Take 1 volume of Anti-IFN gamma-Eu (20 ×) (1V µl) and add it to 19 volumes of Detection Buffer (19V µl). Mix well and set aside.

   Preparation of Anti-IFN gamma-A3 working solution:

   ● Remove Anti-IFN gamma-A3 (20 ×) from the refrigerator and allow it to thaw at room temperature until completely dissolved. Mix thoroughly before use.

   ● Take 1 volume of Anti-IFN gamma-A3 (20 ×) (1V µl) and add it to 19 volumes of Detection Buffer (19V µl). Mix well and set aside.

   ▲ Anti-IFN gamma-Eu (20 ×) and Anti-IFN gamma-A3 (20 ×) are recommended to be aliquoted and stored at -30℃ ~ -15℃, avoiding repeated freeze-thaw cycles.

2. Standard Preparation

   The reaction system for a 96/384-well low volume white plate is 20 µl per well, and each well requires 16 µl Standard. Calculate the required Standard volume before preparation.

   ● Remove the IFN gamma Standard (Lyophilized) from the refrigerator and allow it to equilibrate to room temperature. Reconstitute the IFN gamma Standard (Lyophilized) by adding 400 µl of deionized or distilled water to fully dissolve it, obtaining the IFN gamma Std.

   ● You can refer to the table below for Standard gradient dilution. The volumes listed in the table are for dispensing Diluent Buffer.

   ● Take 60 µl of the reconstituted IFN gamma Std and add it to 120 µl of Diluent Buffer. Mix thoroughly to obtain Std 7.

   ● Take 60 µl Std 7 and add it to 66 µl of Diluent Buffer. Mix thoroughly to obtain Std 6.

   ● Dilute 2.1 times in the same manner to obtain Std 5 to Std 1.

Standard	Dilution Method	Standard Concentration pg/ml
Std 7	60 µl IFN gamma Std + 120 µl Diluent Buffer	4000
Std 6	60 µl Std 7 +66 µl Diluent Buffer	1905
Std 5	60 µl Std 6 +66 µl Diluent Buffer	907
Std 4	60 µl Std 5 +66 µl Diluent Buffer	432
Std 3	60 µl Std 4 +66 µl Diluent Buffer	206
Std 2	60 µl Std 3 +66 µl Diluent Buffer	98
Std 1	60 µl Std 2 +66 µl Diluent Buffer	47
Std 0	60 µl Diluent Buffer	0

   ▲ The mixed IFN gamma Std is recommended to be stored at -85℃ ~ -65℃, avoiding repeated freeze-thaw cycles.

07-2/Sample Preparation

To mitigate the effects of matrix interference in samples, it is recommended to dilute the sample with Diluent Buffer at a dilution factor greater than 2-fold. The specific dilution factor should be determined based on the actual application requirements.

▲ If the sample is diluted with a culture medium, the corresponding calibration curve should also be prepared with the same culture medium.

07-3/Reaction system

1. Sample adding

The reaction volume for the 96/384-well low volume white plate is 20 μl. Adding sample is performed according to the experimental grouping and reaction system outlined in the table below.

	Standard / Samples	Negative Control
Standard / Samples	16 μl	-
Anti-IFN gamma-Eu	2 μl	2 μl
Anti-IFN gamma-A3	2 μl	2 μl
Diluent Buffer	-	16 μl
Detection Buffer	-	-

2. The order of reagent adding is:

- Add 16 μl of Standard/sample to the 96/384-well low volume white plate.

▲ It is recommended to run triplicate measurements for each point on the standard curve and for the samples to improve detection accuracy.

- Mix the Anti-IFN gamma-Eu working solution and the Anti-IFN gamma-A3 working solution in a 1:1 volume ratio. Then add 4 μl of the mixed solution into the reaction system. It is recommended to gently pipette and mix thoroughly in each well gently at least five times.

3. Incubate overnight (recommended 12-16 hours) at room temperature or 25℃, then detected by an microplate reader (with HTRF/TR-FRET module). The excitation light is 320/340 nm, and emission light at two wavelengths (665 nm and 620 nm) are detected.

08/Data Processing

1. Divide the 665 nm fluorescence value by the 620 nm fluorescence value, then multiply the result by 10^4 to obtain the Ratio value ($665/620*10^4$)

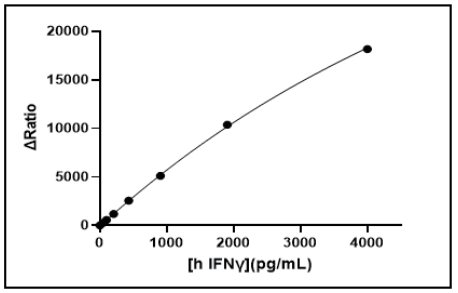
2. Subtract the Ratio value of Standard 0 well from the Ratio value of each well to obtain the ΔRatio value.

3. Perform a 4-parameter fit (weighted $1/y^2$) with IFN gamma concentration as the x-axis and ΔRatio values as the y-axis.

▲ Add a weight of $1/y^2$ to the equation to weight the data, ultimately resulting in a 4PL $1/y^2$ fit. The $1/y^2$ weighting correction taken into account is for the variance change that occurs with increasing signal, thereby improving the accuracy of the calibration curve at low/high concentrations.

4. Substitute the ΔRatio value of the sample into the fitting equation of the calibration curve to calculate the sample concentration. Multiply by the dilution factor to obtain the actual concentration of the sample. If the ΔRatio value of the sample exceeds the range of ΔRatio values of the calibration curve, adjust the dilution factor before conducting the detection. This calibration curve is only for demonstration, and a new calibration curve will be generated during each experiment.

	IFN γ(pg/ml)	Ratio	ΔRatio	CV(%)
Standard 7	4000	18722	18163	0.6%
Standard 6	1905	10942	10383	0.4%
Standard 5	907	5663	5104	1.3%
Standard 4	432	3125	2565	1.6%
Standard 3	206	1747	1188	1.3%
Standard 2	98	1129	570	0.9%
Standard 1	47	825	266	3.0%
Standard 0	0	559	0	1.6%



▲ The differences in detection results between different laboratories and microplate readers may be influenced by variations in experimental conditions and equipment calibration.

09/Product Performance Indexes

09-1/Analytical Sensitivity

Repeat the measurement of Standard 0 twenty times to determine the detection limit, and repeat the measurement of 21 pg/ml twenty times to determine the quantification limit.

	Diluent	DMEM	RPMI
Limit of detection (LOD)	5 pg/ml	31 pg/ml	6 pg/ml
Limit of Quantitation (LOQ)	21 pg/ml		

▲ The differences in detection results between different laboratories and microplate readers may be influenced by variations in experimental conditions and equipment calibration.

09-2/Precision

The reagent kit utilizes five quality control samples of known concentrations add repeated three times on the same ELISA plate to assess within-batch precision. Additionally, five known concentrations of quality control samples are measured by two technicians across six independent analytical batches to evaluate between-batch precision.

Within-batch Precision

Samples	n	Measured average concentration (pg/ml)	SD	CV
Quantitative upper limit concentration sample	3	4182	48.82	1.17%
High-concentration sample	3	2975	178.95	6.02%
Medium-concentration sample	3	1511	39.31	2.60%
Low-concentration sample	3	62	3.58	5.73%
Quantitative lower limit concentration sample	3	24.12	1.27	5.26%

Between-batch Precision				
Samples	n	Measured average concentration (pg/ml)	SD	CV
Quantitative upper limit concentration sample	6	3967	138.60	3.49%
High-concentration sample	6	2781	138.19	4.97%
Medium-concentration sample	6	1418	57.42	4.05%
Low-concentration sample	6	60	3.90	6.53%
Quantitative lower limit concentration sample	6	23.57	1.79	7.59%

09-3/Accuracy

The reagent kit utilizes five known concentrations of quality control samples to repeat the determination three times on the same elisa plate to evaluate the accuracy of the determination concentration to the theoretical concentration ratio.

Samples	n	Theoretical Concentration (pg/ml)	Measured Mean Concentration (pg/ml)	Accuracy % (80-120)
Quantitative upper limit concentration sample	3	4000	4209	105
High-concentration sample	3	2800	3071	110
Medium-concentration sample	3	1500	1527	102
Low-concentration sample	3	60	62	103
Quantitative lower limit concentration sample	3	21	20	94

09-4/Specificity

The reagent kit can detect both native and recombinant human IFN gamma. Specificity testing is conducted for the factors listed below, and no significant cross-reactivity is observed.

Human			
IL1 beta	IL2	IL6	IL8
IL10	GM-CSF	TNF alpha	

09-5/Traceability

NIBSC/WHO (82/587) approximate value (IU/ml) = 0.019 × Human IFNγ value (pg/ml).