Add&Read Human IL2 Quantitative Detection Kit

DD2705

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Instruction for Use

Version 24.1

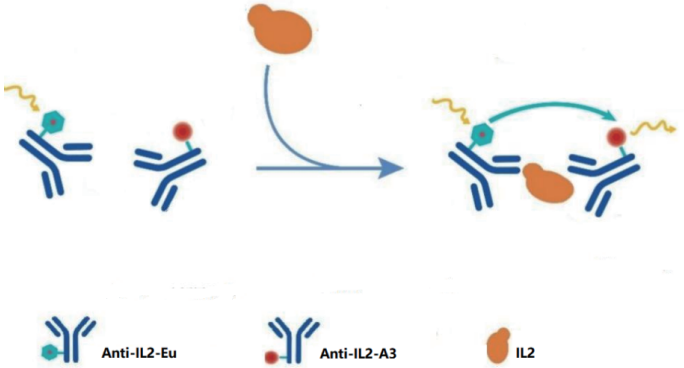
目录 Contents

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

01/Product Description

Interleukin-2 (IL2) is a key immunoregulatory cytokine primarily produced by activated T cells. It plays crucial roles in promoting the proliferation of T lymphocytes and natural killer (NK) cells, stimulating the differentiation and proliferation of B cells, and enhancing antibody production. IL2 is essential in immune responses, immune regulation, and anti-tumor immunity within the body.

This reagent kit utilizes a sandwich method to detect IL2 levels. The kit contains IL2 Standard and two monoclonal antibodies specific to IL2, one coupled with Eu (donor, Anti-IL2-Eu) and the other coupled with A3 (acceptor, Anti-IL2-A3). When both antibodies bind to IL2 simultaneously, the proximity of Anti-IL2-Eu and Anti-IL2-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 IL2 concentration in the sample is proportional to the FRET signal (ratio of fluorescence intensity at 665 nm to 620 nm).



02/Product Components

Components	DD2705-01(96 tests)	DD2705-02(500 tests)	DD2705-03(10,000 tests)
IL2 Standard	400 µl	2 × 400 µl	4 × 400 µl
Anti-IL2-Eu (20 ×)	12 µl	50 µl	1 ml
Anti-IL2-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-IL2-Eu (20 ×) and Anti-IL2-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 IL2 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-IL2-Eu and Anti-IL2-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-IL2-Eu and 2 μl of Anti-IL2-A3 working solutions to each 20 μl system. Before preparing, calculate the required volumes of Anti-IL2-Eu (20 ×) and Anti-IL2-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-IL2-Eu working solution:

   ● Remove Anti-IL2-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-IL2-Eu (20 ×) (1V μl) and add it to 19 volumes of Detection Buffer (19V μl). Mix well and set aside.

   Preparation of Anti-IL2-Eu working solution:

   ● Remove Anti-IL2-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-IL2-A3 (20 ×) (1V μl) and add it to 19 volumes of Detection Buffer (19V μl). Mix well and set aside.

   ▲ Anti-IL2-Eu (20 ×) and Anti-IL2-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 IL2 Standard from the refrigerator and allow it to equilibrate to room temperature and mix to obtain an IL2 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 IL2 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 72 μl of Diluent Buffer. Mix thoroughly to obtain Std 6.

   Dilute 2.2 times in the same manner to obtain Std 5 to Std 1.

Standard	Dilution Method	Standard Concentration pg/ml
Std 7	60 μl IL2 Std + 120 μl Diluent Buffer	8000
Std 6	60μl Std 7 + 72 μl Diluent Buffer	3636
Std 5	60 μl Std 6 +72μl Diluent Buffer	1653
Std 4	60 μl Std 5 + 72 μl Diluent Buffer	751
Std 3	60 μl Std 4 + 72 μl Diluent Buffer	342
Std 2	60 μl Std 3 + 72 μl Diluent Buffer	155
Std 1	60 μl Std 2 + 72 μl Diluent Buffer	71
Std 0	72 μl Diluent Buffer	0

▲ The mixed IL2 Std is 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-IL2-Eu	2 µl	2 µl
Anti-IL2-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-IL2-Eu working solution and the Anti-IL2-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 at room temperature or 25℃ for 2 hours, then detected by an microplate reader (with HTRF/TR-FRET module). The excitation light is 320/340 nm, and emission light at two wavelengths (620 nm and 665 nm) are detected.

08/Data Processing

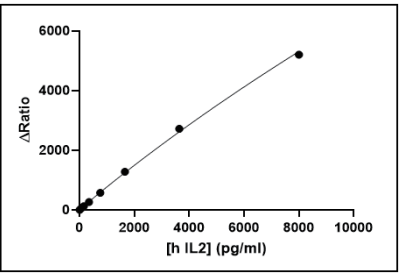
1. Divide the 665 nm fluorescence value by the 620 nm fluorescence value, then multiply the result by 10^4 to obtain the Ratio value (665/620*10^4)
2. Subtract the Ratio value of Standard 0 well from the Ratio value of each well to obtain the ΔRatio value.
3. Perform a 4-parameter fit (weighted $1/y^2$) with IL2 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 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.

09/Product Performance Indexes

	IL2 (pg/ml)	Ratio	ΔRatio	CV(%)
Standard7	8000	5301	5201	1.6%
Standard6	3636	2815	2716	1.2%
Standard5	1653	1379	1279	1.4%
Standard4	751	673	574	0.6%
Standard3	342	362	263	0.6%
Standard2	155	224	124	2.7%
Standard1	71	162	62	3.5%
Standard0	0	100	0	1.5%



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

09-1/Analytical Sensitivity

Repeat the measurement of Standard 0 twenty times to determine the detection limit, and repeat the measurement of 37 pg/ml twenty times to determine the quantification limit.

	Diluent	DMEM	RPMI
Limit of detection (LOD)	5.64 pg/ml	24.74 pg/ml	6.81 pg/ml
Limit of Quantitation (LOQ)	37 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 white 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	7565	298.04	3.94%
High-concentration sample	3	6526	481.46	7.38%
Medium-concentration sample	3	1234	38.06	3.08%
Low-concentration sample	3	210	3.36	1.60%
Quantitative lower limit concentration sample	3	67	2.02	3.02%

Between-batch Precision				
Samples	n	Measured average concentration (pg/ml)	SD	CV
Quantitative upper limit concentration sample	6	7629	210.20	2.76%
High-concentration sample	6	6288	424.62	6.75%
Medium-concentration sample	6	1175	71.95	6.12%
Low-concentration sample	6	208	5.37	2.58%
Quantitative lower limit concentration sample	6	71	5.05	7.16%

09-3/Accuracy

The reagent kit utilizes five known concentrations of quality control samples to repeat the determination three times on the same white 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	8000	7565	95
High-concentration sample	3	6400	6526	102
Medium-concentration sample	3	1200	1234	103
Low-concentration sample	3	200	210	105
Quantitative lower limit concentration sample	3	71	67	95

09-4/Specificity

The reagent kit can detect both native and recombinant human IL2. Specificity testing is conducted for the factors listed below, and no significant cross-reactivity is observed.

Human			
IL1 beta	IFN gamma	IL6	IL8
IL10	GM-CSF	TNF alpha	

09-5/Traceability

NIBSC/WHO (86/500) approximate value (IU/ml) = 0.01 × Human IL2 value (pg/ml).