Add&Read Human IL6 Quantitative Detection Kit(Customized)

DD2703-C

Vazyme

Instruction for Use

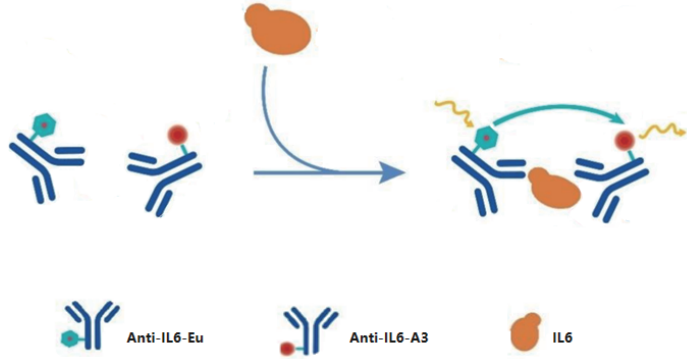
Version 24.2

Contents

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

01/Product Description

Interleukin-6 (IL6) is a pleiotropic cytokine mainly produced by macrophages, T lymphocytes, and B lymphocytes. It regulates the growth, differentiation, immune responses, acute-phase reactions, and hematopoietic functions of various cells, and plays an important role in the body's immune response to infections. IL6 plays a central role in acute inflammatory reactions, and serum IL6 levels can increase in various infectious diseases. IL6 activation has a short duration, quick decline during recovery, and a large magnitude, making it an excellent indicator for assessing early inflammation, sepsis, infection severity, and prognosis. This reagent kit utilizes a sandwich method to detect IL6 levels. The kit contains IL6 Standard and two monoclonal antibodies specific to IL6, one coupled with Eu (donor, Anti-IL6-Eu) and the other coupled with A3 (acceptor, Anti-IL6-A3). When both antibodies bind to IL6 simultaneously, the proximity of Anti-IL6-Eu and Anti-IL6-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 IL6 concentration in the sample is proportional to the FRET signal (ratio of fluorescence intensity at 665 nm to 620 nm).



02/Product Components

Components	DD2703-C-01(96 tests)	DD2703-C-02(500 tests)	DD2703-C-03(10,000 tests)
IL6 Standard (Lyophilized)	1 Vial	2 Vial	4 Vial
Anti-IL6-Eu (20 ×)	12 µl	50 µl	1 ml
Anti-IL6-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	5 ml	100 ml

03/Storage Conditions

The reagent kit should be stored at -30 ~ -15℃ and transported at ≤0℃.

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-IL6-Eu (20 ×) and Anti-IL6-A3 (20 ×) are recommended to be aliquoted and stored at -30 ~ -15℃ in the stock solution (20 ×), avoiding repeated freeze-thaw cycles. The aliquot volume is suggested to be more than 10 µl.
2. The reconstituted IL6 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 precipatation 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-IL6-Eu and Anti-IL6-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-IL6-Eu and 2 µl of Anti-IL6-A3 working solutions to each 20 µl system. Before preparing, calculate the required volumes of Anti-IL6-Eu (20 ×) and Anti-IL6-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-IL6-Eu working solution:

   ● Remove Anti-IL6-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-IL6-Eu (20 ×) (1V µl) and add it to 19 volumes of Detection Buffer (19V µl). Mix well and set aside.

Preparation of Anti-IL6-A3 working solution:

● Remove Anti-IL6-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-IL6-A3 (20 ×) (1V μl) and add it to 19 volumes of Detection Buffer (19V μl). Mix well and set aside.

▲ Anti-IL6-Eu (20 ×) and Anti-IL6-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 shallow well plate is 20 μl per well, and each well requires 16 μl Standard. Calculate the required Standard volume before preparation.

● Remove the IL6 Standard (Lyophilized) from the refrigerator and allow it to equilibrate to room temperature. Reconstitute the IL6 Standard (Lyophilized) by adding 400 μl of deionized or distilled water to fully dissolve it, obtaining the IL6 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 IL6 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 140 μl of Diluent Buffer. Mix thoroughly to obtain Std 6.

● Dilute 2.4 times in the same manner to obtain Std 5 to Std 1.

Standard	Dilution Method	Standard Concentration pg/ml
Std 7	60 μl IL6 Std + 120 μl Diluent Buffer	8500
Std 6	60 μl Std 7 + 84 μl Diluent Buffer	3542
Std 5	60 μl Std 6 + 84 μl Diluent Buffer	1476
Std 4	60 μl Std 5 + 84 μl Diluent Buffer	615
Std 3	60 μl Std 4 + 84 μl Diluent Buffer	256
Std 2	60 μl Std 3 + 84 μl Diluent Buffer	107
Std 1	60 μl Std 2 + 84 μl Diluent Buffer	44
Std 0	84 μl Diiuent Buffer	0

▲ The reconstituted TNF alpha 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 ≥ 2-fold. The specific dilution factor should be determined based on the actual application requirements.

▲ If the sample is diluted with a culture medium, then 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.

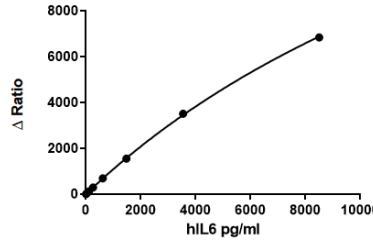
	Standard / Samples	Negative Control
Standard / Samples	16 μl	-
Anti-TNF alpha-Eu	2 μl	2 μl
Anti-TNF alpha-A3	2 μl	2 μl
Diluent Buffer	-	16 μl
Detection Buffer	-	-

2. The order of reagent adding is:
   - Add 16 μl of Standard/sample to the reaction system (96/384-well low volume white plate).
   - Mix the Anti-IL6-Eu working solution and the Anti-IL6-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 twice.
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 (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 IL6 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.

	IL6 (pg/ml)	Ratio	ΔRatio	CV (%)
Standard 7	8500	6974	6838	2%
Standard 6	3542	3645	3509	1%
Standard 5	1476	1684	1548	3%
Standard 4	615	827	691	3%
Standard 3	256	425	289	1%
Standard 2	107	259	123	1%
Standard 1	44	187	51	1%
Standard 0	0	136	0	2%



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 Standard 1 twenty times to determine the quantification limit.

	Diluent	DMEM	RPMI
Limit of detection (LOD)	6.6 pg/ml	17.2 pg/ml	20.5 pg/ml
Limit of Quantitation (LOQ)	35 pg/mll		

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	8110	369	5%
High-concentration sample	3	6753	338	5%
Medium-concentration sample	3	738	31	4%
Low-concentration sample	3	105	6	6%
Quantitative lower limit concentration sample	3	34	3	8%

Between-batch Precision

Samples	n	Measured average concentration (pg/ml)	SD	CV
Quantitative upper limit concentration sample	6	7972	311	4%
High-concentration sample	6	7011	374	5%
Medium-concentration sample	6	716	33	5%
Low-concentration sample	6	106	7	6%
Quantitative lower limit concentration sample	6	35	3	10%

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	8166 7702 7582	7817	92	89-96
High-concentration sample	7338 6332 6664	6778	97	90-105
Medium-concentration sample	756 707 750	738	105	101-108
Low-concentration sample	107 104 105	105	105	104-107
Quantitative lower limit concentration sample	37 36 31	35	100	89-106

09-4/Specificity

The reagent kit can detect both native and recombinant human IL6. Specificity testing is conducted for the factors listed below, and no significant cross-reactivity is observed.

Human			
IL1 beta	IL8	IL10	IL2
CXCL-10	IFN gamma	GM-CSF	TNF alpha

09-5/Traceability

NIBSC/WHO (89/548) approximate value (IU/ml) = 0.1 × Human IL6 value (pg/ml).

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