Add & Read Human IL7 Quantitative Detection Kit

DD2711

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User Manual

Version 25.1

Vazyme

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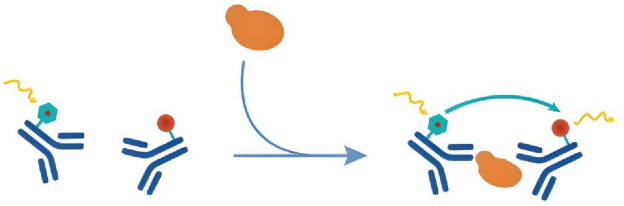
01/Product Description

Interleukin-7 (IL-7) is a pleiotropic cytokine primarily produced by bone marrow stromal cells. Its main functions include promoting the growth of B and T cells and exerting anti-apoptotic effects. It plays a crucial role in the growth, survival, and differentiation of T cells, while also exhibiting potential chemotactic activity. Like other cytokines, IL-7 is an immunoregulatory protein that plays an important role in the normal development of the immune system and the maintenance of normal immune function.

This kit uses a sandwich assay to detect IL-7 levels. The kit includes IL-7 Standard and two monoclonal antibodies specifically recognizing IL-7. One antibody is conjugated with Eu (donor, Anti-IL-7-Eu), and the other is conjugated with A3 (acceptor, Anti-IL-7-A3). When both antibodies bind to IL-7 simultaneously, Anti-IL-7-Eu and Anti-IL-7-A3 come into close proximity, enabling fluorescence resonance energy transfer (FRET). The fluorescence donor is excited by light at 320/340 nm, emitting light at 620 nm. This 620 nm light then excites the fluorescence acceptor, which emits light at 665 nm. The concentration of IL-7 in the sample is proportional to the FRET signal value (the ratio of light intensity at 665 nm to 620 nm).



02/Components

Cap	Components	DD2711-01 (96 tests)	DD2711-02 (500 tests)	DD2711-03 (10,000 tests)
	IL7 Standard	400 µl	2 × 400 µl	4 × 400 µl
■	Anti-IL7-Eu (20 ×)	12 µl	50 µl	1 ml
■	Anti-IL7-A3 (20 ×)	12 µl	50 µl	1 ml
	Detection Buffer (ready-to-use)	500 µl	3 ml	50 ml
	Diluent Buffer (ready-to-use)	5 ml	10 ml	100 ml

03/Storage Conditions and Validity Period

Store at -30℃ ~ -15℃ and transport 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-IL7-Eu (20 ×) and Anti-IL7-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 IL7 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-IL7-Eu and Anti-IL7-A3 working solutions (stock solution is 20 ×)
For areactionvolumeof 20µlin a96/384-welllow volume white plate,itis recommended to add 2 µl of Anti-IL7-Eu and 2 µl of Anti-IL7-A3 working solutions to each 20 µl system. Before preparing, calculate the required volumes of Anti-IL7-Eu (20 ×) and Anti -IL7-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-IL7-Eu working solution:
Remove Anti-IL7-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-IL7-Eu (20 ×) (1V µl) and add it to 19 volumes of Detection Buffer (19V µl). Mix well and set aside.

Preparation of Anti-Il7-A3 working solution:
Remove Anti-IL7-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-IL7-A3 (20 ×) (1V µl) and add it to 19 volumes of Detection Buffer (19V µl). Mix well and set aside.

▲Anti-IL7-Eu (20 ×) and Anti-IL7-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.

a.Reconstitute the IL7 Standard (Lyophilized) obtaining the IL7 Std.

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

c.Take 60 µl of the reconstituted IL7 Std and add it to 120 µl of Diluent Buffer. Mix thoroughly to obtain Std 7.

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

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

Standard	Dilution Method	Standard Concentration pg/ml
Standard 7	60 µl IL7 Standard + 120 µl Diluent Buffer	4000
Standard 6	60 µl Standard 7 + 66 µl Diluent Buffer	1905
Standard 5	60 µl Standard 6 + 66 µl Diluent Buffer	907
Standard 4	60 µl Standard 5 + 66 µl Diluent Buffer	432
Standard 3	60 µl Standard 4 + 66 µl Diluent Buffer	206
Standard 2	60 µl Standard 3 + 66 µl Diluent Buffer	98
Standard 1	60 µl Standard 2 + 66 µl Diluent Buffer	47
Standard 0	66 µl Diluent Buffer	0

▲The mixed IL7 Std is recommended to be stored at -85 ~ -65, avoiding repeated freeze-thaw cycles.

07-2/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-IL7-Eu	2 µl	2 µl
Anti-IL7-A3	2 µl	2 µl
Diluent Buffer	-	16 µl
Detection Buffer	-	-

2.The order of reagent adding is:

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

b.Mix the Anti-IL7-Eu working solution and the Anti-IL7-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.

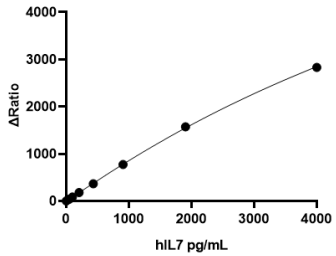
3. Incubate for 1 hours at room temperature or 25℃, then detected by an plate reader (with HTRF/TR-FRET module). The excitation light is 320/340 nm, and emission light at two wavelengths (665 nm and 620 nm) are detected

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 IL7 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 theΔRatio 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.

	IL7 (pg/ml)	Ratio	ΔRatio	CV (%)
Standard 7	4000	2952	2829	1.2
Standard 6	1905	1695	1571	0.9
Standard 5	907	896	773	2.8
Standard 4	432	490	366	2.2
Standard 3	206	303	179	1.8
Standard 2	98	207	83	2.4
Standard 1	47	162	38	1.4
Standard 0	0	124	0	0.5



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 35 pg/ml twenty times to determine the quantification limit.

	Diluent	DMEM	RPMI
Limit of detection (LOD)	9 pg/ml	23 pg/ml	17 pg/ml
Limit of Quantitation (LOQ)	35 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.

Table1.Within-batch Precision

Samples	n	Mean concentration (pg/ml)	SD	CV (%)
ULOQ	3	3826	86.79	2.27
High Concentration Sample	3	2753	64.75	2.35
Medium Concentration Sample	3	1449	15.25	1.05
Low Concentration Sample	3	63	3.57	5.70
LLOQ	3	37	1.19	7.39

Table2.Between-batch Precision

Samples	n	Mean concentration (pg/ml)	SD	CV (%)
ULOQ	6	3868	110.38	2.85
High Concentration Sampl	6	2830	119.60	4.23
Medium Concentration Sample	6	1480	30.69	2.07
Low Concentration Sample	6	65	1.74	2.69
LLOQ	6	34	1.89	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	Concentration (pg/ml)	Mean concentration (pg/ml)	Accuracy %	Accuracy Range %
ULOQ	3761	3826	95.66	94.04 - 98.12
	3925			
	3792			
High Concentration Sample	2691	2753	98.33	96.10 - 100.72
	2820			
	2749			
Medium Concentration Sample	1463	1449	96.63	95.52 - 97.53
	1433			
	1452			
Low Concentration Sample	60	63	104.44	99.24 - 110.93
	62			
	67			
LLOQ	38	37	106.07	102.66 - 109.47
	37			
	36			

09-4/Specificity

The reagent kit can detect both native and recombinant human IL7. Specificity testing is conducted for the factors listed below, and no significant cross-reactivity is observed.

Human			
IL2	IL4	IL9	IL15
IL-21	GM-CSF	IFN gamma	

09-5/Calibration

This Add & Read® is calibrated against a highly purified *E. coli*-expressed recombinant human IL-7 produced at Vazyme Systems®