Add&Read Human IL8 Quantitative Detection Kit

DD2709

Vazyme

Instruction for Use

Version 24.1

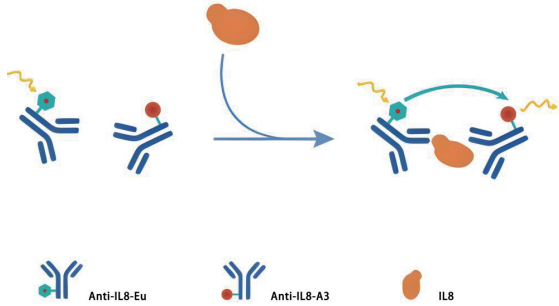
Contents

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

01/ Product Description

Interleukin8 (IL8), also known as CXCL8, is a widely expressed pro-inflammatory member of the CXC family of chemokines, initially identified as a leukocyte chemoattractant. It is secreted by a variety of cell types, including monocytes, macrophages, neutrophils, eosinophils, T lymphocytes, epithelial cells, and fibroblasts. It acts as a chemoattractant by directing neutrophils to sites of infection, thereby modulating its regulation of the inflammatory response.

This kit uses a sandwich method to detect IL8 levels. The kit contains IL8 Standard and two monoclonal antibodies that specifically recognize IL8. One coupled with Eu (donor, Anti-IL8-Eu), and the other coupled with A3 (acceptor, Anti-IL8-A3). When both antibodies bind to IL8 simultaneously, Anti-IL8-Eu and Anti-IL8-A3 come close together, leading to fluorescent resonance energy transfer (FRET). 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 concentration of IL8 in the sample is proportional to the FRET signal value (the ratio of fluorescence intensity at 665 nm to 620 nm).



02/Components

Components	DD2709-01 (96 tests)	DD2709-02 (500 tests)	DD2709-03 (10,000 tests)
IL8 Standard (Lyophilized)	1 Vial	2 × 1 Vial	4 × 1 Vial
Anti-IL8-Eu (20 ×)	12 µl	50 µl	1 ml
Anti-IL8-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

03/Storage

Store at -30 ~ -15℃ and ship at ≤0℃.

▲ IL8 Standard, Anti-IL8-Eu and Anti-IL8-A3 should be stored at -30 ~ -15℃. For the first use, please dispense as needed and avoid repeated freezing and thawing.

▲ Detection Buffer and Diluent Buffer should be stored at -30 ~ 8℃.

04/Applications

This product is suitable for cell supernatants.

05/Self-prepared Materials

96/384-well low volume white plate;
Microplate reader (equipped with HTRF/TR-FRET module).

06/Notes

1. Anti-IL8-Eu (20 ×) and Anti-IL8-A3 (20 ×) are recommended to be aliquoted and stored at -30 ~ -15°C in the stock solution (20 ×), avoiding repeated freeze-thaw cycles. The aliquot volume is suggested to bo more than 10 μl.
2. The resuspended IL8 Standard is recommended to be stored at -85 ~ -65°C, 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 gentle 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-IL8-Eu and Anti-IL8-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-IL8-Eu and 2 μl of Anti-IL8-A3 working solutions to each 20 μl system. Before preparing, calculate the required volumes of Anti-IL8-Eu (20 ×) and Anti-IL8-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-IL8-Eu working solution:
   a. Remove Anti-IL8-Eu (20 ×) from the refrigerator and allow it to thaw at room temperature until completely dissolved. Mix thoroughly before use.
   b. Take 1 volume of Anti-IL8-Eu (20 ×) (1 V μl) and add it to 19 volumes of Detection Buffer (19 V μl). Mix well and keep it on standby.

Preparation of Anti-IL8-A3 working solution:

a. Remove Anti-IL8-A3 (20 ×) from the refrigerator and allow it to thaw at room temperature until completely dissolved. Mix thoroughly before use.

b. Take 1 volume of Anti-IL8-A3 (20 ×) (1 V µl) and add it to 19 volumes of Detection Buffer (19 V µl). Mix well and keep it on standby.

▲ Anti-IL8-Eu (20 ×) and Anti-IL8-A3 (20 ×) are recommended to be aliquoted and stored at -30 ~ -15°C, 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. Take the IL8 Standard (Lyophilized) out from the refrigerator and let it return to room temperature. Resuspend the IL8 Standard (Lyophilized) by adding 400 µl of deionized or distilled water to fully dissolved it, obtaining the IL8 Standard.

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 40 µl of the dissolved IL8 Standard and add it to 80 µl of Diluent Buffer. Mix thoroughly to obtain Standard 7.

d. Take 60 µl Standard 7 and add it to 72 µl of Diluent Buffer. Mix thoroughly to obtain Standard 6.

e. Dilute 2.2-fold in the same manner to obtain Standard 5 to Standard 1.

Standard	Dilution Method	Standard Concentration pg/ml
Standard 7	40 µl IL8 Standard + 80 µl Diluent Buffer	4,000
Standard 6	60 µl Standard 7 + 72 µl Diluent Buffer	1,818.2
Standard 5	60 µl Standard 6 + 72 µl Diluent Buffer	826.4
Standard 4	60 µl Standard 5 + 72 µl Diluent Buffer	375.7
Standard 3	60 µl Standard 4 + 72 µl Diluent Buffer	170.8
Standard 2	60 µl Standard 3 + 72 µl Diluent Buffer	77.6
Standard 1	60 µl Standard 2 + 72 µl Diluent Buffer	35.3
Standard 0	72 µl Diluent Buffer	0

▲ The resuspended IL8 Standard should be aliquoted and stored at -85 ~ -65°C, avoiding repeated freeze-thaw cycles.

07-2/Reaction System

1. Sample Adding

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

	Standard/Samples	Negative Control
Standard/Samples	16 µl	-
Anti-IL8-Eu	2 µl	2 µl
Anti-IL8-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 reaction system (96/384-well low volume white plate).

   b. Mix the Anti-IL8-Eu working solution and the Anti-IL8-A3 working solution in a 1:1 volume ratio until. Then add 4 µl of the mixed solution into the reaction system. It is recommended to gently pipette and mix thoroughly in each well solution gently at least twice.

3. Incubate at room temperature or 25°C for 1 h, then detect by a microplate reader (equipped with an 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 ($665/620 \times 10^4$).

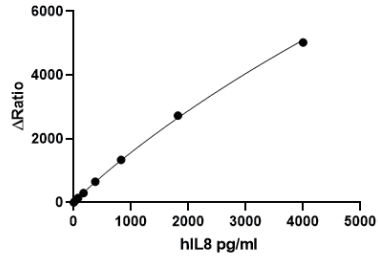
2. Subtract the Ratio value of Standard 0 well from the Ratio value of each well to obtain the ΔRatio value.

3. Perform a 4-parameter fit (weighted by $1/y^2$) with the IL8 concentration as the x-axis and the ΔRatio value as the y-axis.

   ▲ Adding $1/y^2$ to the equation weight the data, ultimately resulting in a 4PL $1/y^2$ fit. The $1/y^2$ weighting correction takes into account the variance changes that occur with increasing signal, and after weighting, it improves the precision of the calibration curve at low/high concentrations.

4. Substitute the sample's ΔRatio value 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 sample's ΔRatio value exceeds the ΔRatio range 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.

	IL8 (pg/ml)	Ratio	ΔRatio	CV (%)
Standard 7	4,000	5,484	5,020	2
Standard 6	1,818.2	3,190	2,726	1
Standard 5	826.4	1,803	1,338	7
Standard 4	375.7	1,119	654	4
Standard 3	170.8	759	295	5
Standard 2	77.6	606	142	7
Standard 1	35.3	538	74	4
Standard 0	0	465	0	5



09/Product Performance Indexes

09-1/Analysis Sensitivity

Repeat the measurement of Standard 0 twenty times to determine the detection limit, and repeat the measurement of 32 pg/ml twenty times to determine the quantification limit.

	Diluent	DMEM	RPMI
Limit of detection (LOD)	13 pg/ml	28 pg/ml	5 pg/ml
Limit of Quantitation (LOQ)	32 pg/ml		

▲ Results may vary due to changes in experimental conditions and Microplate reader.

09-2/Precision

The reagent kit utilizes five quality control samples of known concentrations repeated three times on the same 96/384-well low volume 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.

Table1. Within-batch Precision

Samples	n	Measured average concentration (pg/ml)	SD	CV (%)
Quantitative upper limit concentration sample	3	3,993	45.1	1.10
High-concentration sample	3	3,156	62	2.00
Medium-concentration sample	3	393	9.5	2.40
Low-concentration sample	3	101	2.9	2.80
Quantitative lower limit concentration sample	3	33	4.5	13.80

Table2. Between-batch Precision

Samples	n	Measured average concentration (pg/ml)	SD	CV (%)
Quantitative upper limit concentration sample	6	4,000	86.3	2.20
High-concentration sample	6	3,180	95.7	3.00
Medium-concentration sample	6	402	15.8	3.90
Low-concentration sample	6	101	9.5	9.40
Quantitative lower limit concentration sample	6	32	4.5	14.10

09-3/Accuracy

The reagent kit utilizes five known concentrations of quality control samples to repeat the determination three times on the same 96/384-well low volume white plate to evaluate the accuracy of the determination concentration to the theoretical concentration ratio.

Samples	Measured Concentration (pg/ml)	Measured Mean Concentration (pg/ml)	Accuracy (%)	Accuracy Range (%)
Quantitative upper limit concentration sample	3,898 3,947 3,939	3,928	98	97 - 99
High-concentration sample	3,088 3,175 3,056	3,106	97	96 - 99
Medium-concentration sample	389 382 406	392	98	97 - 101
Low-concentration sample	89 92 85	89	89	92 - 95
Quantitative lower limit concentration sample	26 27 32	28	88	80 - 100

09-4/Specificity

This kit can recognize both natural and recombinant human IL8. Specificity testing is conducted for the factors listed below, and no significant cross-reactivity is observed.

Human			
IL1 beta	IL2	IL6	IL10
CXCL-10	TNF alpha	IFN gamma	

09-5/Traceability

NIBSC/WHO (89/520) approximate value (IU/ml) = 0.01 × Human IL8 value (pg/ml).

Vazyme Biotech Co.,Ltd.
www.vazyme.com
400-007-8058 (China) +86 400-168-5000 (Global)
support@vazyme.com