FRT-P072-EN.01

Human IL-21 / IL-21R Inhibitor Screening Kit (TR-FRET)

Pack Size: 100 tests & 500 tests

Catalog Number： FRT-P072

IMPORTANT: Please read this manual carefully before performing your experiment.

For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedure

PRODUCT OVERVIEW

This Human IL-21 / IL-21R Inhibitor Screening Kit (TR-FRET) is based on a homogeneous (no wash) competition TR-FRET technology (Time-Resolved Fluorescence Resonance Energy Transfer) to screen for inhibitors of human IL-21 binding to human IL-21 R within one hour. It can also be used as a universal detection tool to identify the ability of human IL-21 to bind to human IL-21 R.

PRINCIPLE OF THE ASSAY

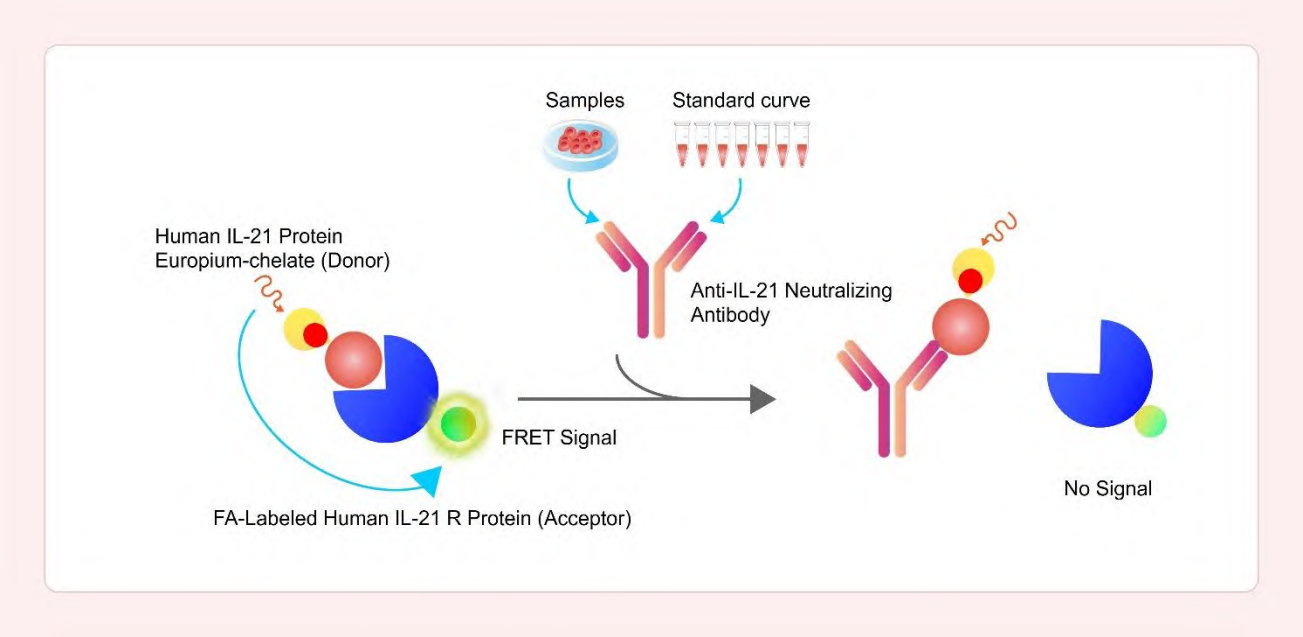
This Human IL-21 / IL-21R Inhibitor Screening Kit (TR-FRET) is based on TR-FRET technology (Time-Resolved Fluorescence Resonance Energy Transfer). This assay uses the mixture of biotinylated human IL-21 and Europium-chelate labeled streptavidin as the Donor and FA Labeled Human IL-21 R Protein as the Acceptor.

— When the sample does not contain the inhibitors that block the binding of human IL-21 to human IL-21 R, the Donor and Acceptor are in close proximity because of the binding of human IL-21 and human IL-21 R. Upon Donor excitation with light of a specific wavelength (337 nm), in addition to Donor emission (620 nm), non-radiative transfer of energy occurs between Donor and Acceptor, resulting in Acceptor emission (665 nm).

— When the sample contains the inhibitors that block the binding of human IL-21 to human IL-21 R, the components inhibit the binding between the Donor and Acceptor and thereby prevent FRET from occurring.

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FIGURE 1. PRINCIPLE OF THE ASSAY



MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (100 tests)	Size (500 tests)	Format	Storage	
					Unopened	Opened
FRTP072-C01	Human IL-21 Protein Europium-chelate	100 tests	500 tests	Powder	2-8°C, protected from light	-70°C, protected from light
FRTP072-C02	FA-Labeled Human IL-21 R Protein	100 tests	500 tests	Powder	2-8°C, protected from light	-70°C, protected from light
FRTP072-C03	Anti-IL-21 Neutralizing Antibody	30 μg	150 μg	Powder	2-8°C	-70°C
DB-04	TR-FRET Sample Dilution Buffer, pH7.4	50 mL	50 mL	Liquid	2-8°C	2-8°C
DB-05	TR-FRET Detection Buffer, pH7.4	50 mL	50 mL	Liquid	2-8°C	2-8°C

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STORAGE AND VALIDITY INSTRUCTIONS

1. Store the unopened kit at 2–8 °C upon receipt.

2. Locate the expiration date on the outer packaging and do not use reagents beyond their expiration date.

3. The opened kit should be stored according to the storage conditions listed in the Components Table. The shelf life of the opened kit is 30 days from the date of opening.

MATERIALS REQUIRED BUT NOT PROVIDED

TABLE 2. MATERIALS REQUIRED BUT NOT PROVIDED

Items	Specifications	Recommendation
Single channel and multi-channel pipettes	Must be calibrated pipettes, with 10 µL, 200 µL and 1000 µL precision	Different pipettes have different levels of precision. Please choose pipettes with appropriate precision.
Pipette tips	Low adsorption pipette tips, all tips need to fit the pipettes	-
96 or 384-well white plate	Non-transparent 96 or 384-well low volume white plates typically give the lowest background signal.	For example, 384-well white plate (iSTAR, Cat. No. GT247.008)
Microplate shaker	For plate shaking	-
EP tubes	For dilution of samples	-
Microplate reader	Plate reader capable of measuring signals at 665 nm/620 nm in TR-FRET mode	For example, BMG LABTECH CLARIOstar® Plus; TECAN Spark®, Infinite® F Nano+, Infinite® F Plex
Timer	-	-
Deionized or distilled water	For reconstitution	-

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REAGENT PREPARATION

1. Bring all reagents and samples up to room temperature (20°C-25°C) before use.

2. Reconstitute the lyophilized materials with deionized or distilled water as described in Table 3 to prepare stock solutions. Allow the solutions to solubilize for 15-30 minutes at room temperature with occasional gentle mixing by inverting the tube 2-3 times. Avoid vigorous shaking or vortexing. The reconstituted stock solutions should be stored at –70°C. It is recommended not to freeze–thaw more than twice.

Note: Both the Human IL-21 Protein Europium-chelate and the FA-Labeled Human IL-21 R Protein stock solutions are light-sensitive and should be protected from light.

TABLE 3. RECONSTITUTION METHODS FOR 100 TESTS AND 500 TESTS

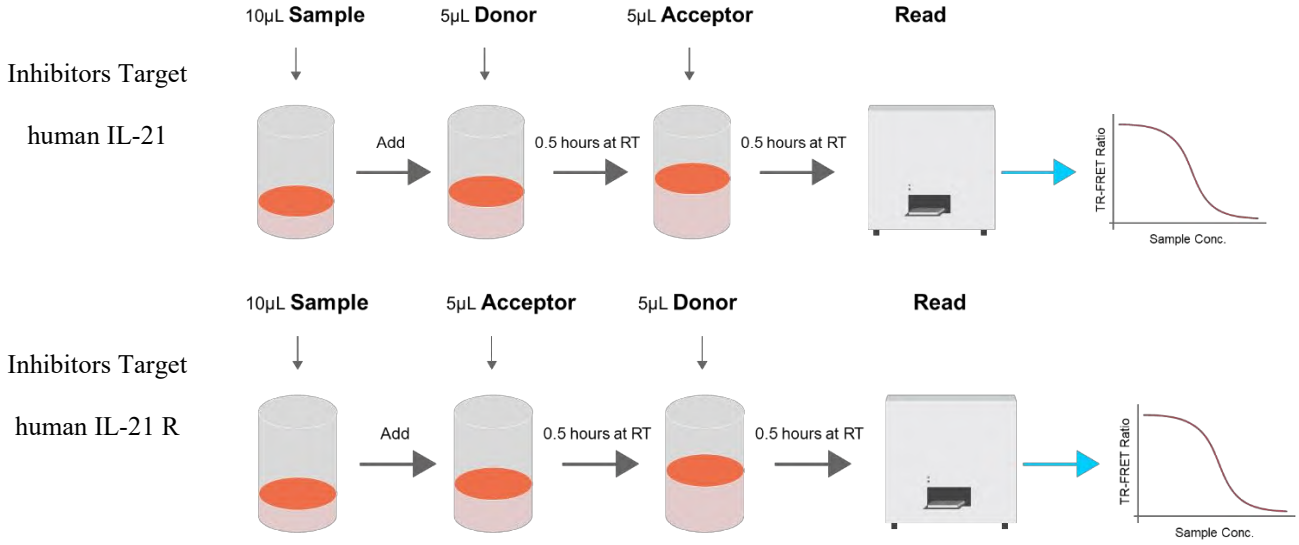
Catalog	Components	Size (100 tests)		Size (500 tests)		Stock Solution Conc.
		Amount	Reconstitution Buffer and Vol.	Amount	Reconstitution Buffer and Vol.	
FRTP072-C01	Human IL-21 Protein Europium-chelate	100 tests	12 µL water	500 tests	60 µL water	/
FRTP072-C02	FA-Labeled Human IL-21 R Protein	100 tests	12 µL water	500 tests	60 µL water	/
FRTP072-C03	Anti-IL-21 Neutralizing Antibody	30 µg	60 µL water	150 µg	300 µL water	500 µg/mL

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RECOMMENDED PROTOCOL

Depending on the intended target of the inhibitors, refer to Figure 2 for the reagent addition sequence.

FIGURE 2. QUICK GUIDE OF THE EXPERIMENTAL WORKFLOW



Note: The following procedure applies to the detection of inhibitors targeting human IL-21. For inhibitors targeting human IL-21 R, add the Sample and Acceptor reagent (FRTP072-C02) first, followed by Donor (FRTP072-C01). Ensure that the inhibitors are incubated with their intended target first for 30 minutes to enhance their inhibitory effect. (Please start with a 30 minutes incubation. If the result is not ideal, please test different time gradients.)

1. Preparation of Sample

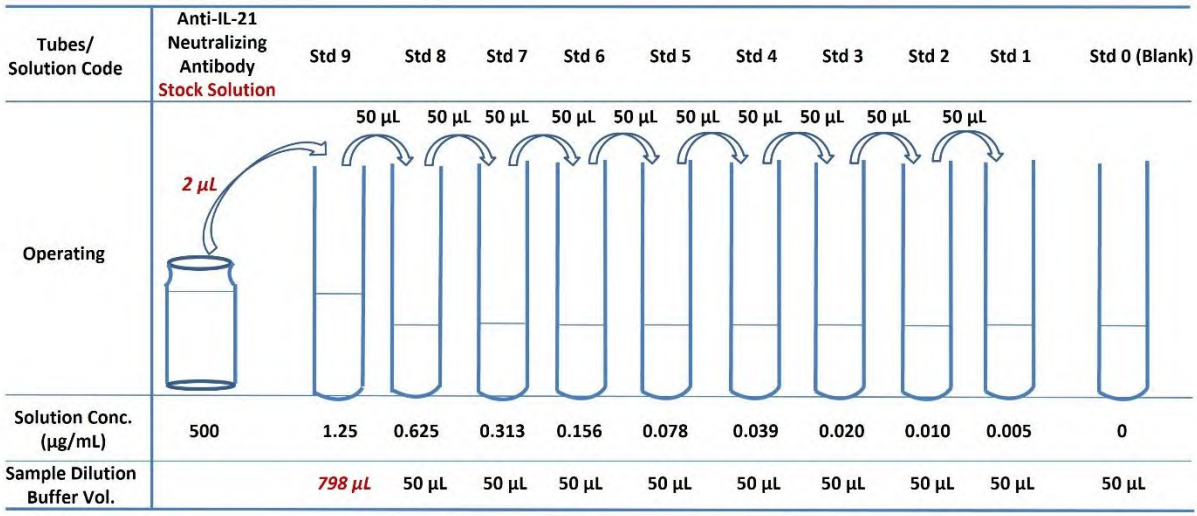
1.1 Dilute sample appropriately using TR-FRET Sample Dilution Buffer, pH7.4 (DB-04).

1.2 If you intend to use the provided **Anti-IL-21 Neutralizing Antibody (FRTP072-C03)** as a standard (Std.), dilute it using TR-FRET Sample Dilution Buffer, pH7.4 (DB-04) as described in Figure 3.

Note: Use DB-04 exclusively as the Sample Dilution Buffer. Do not use TR-FRET Detection Buffer, pH7.4 (DB-05) or any buffers from external sources.

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FIGURE 3. PREPARATION OF SERIAL DILUTIONS OF THE ANTI-IL-21 NEUTRALIZING ANTIBODY



2. Preparation of Donor working solution

Dilute the **Human IL-21 Protein Europium-chelate (FRTP072-C01)** stock solution 50-fold with **TR-FRET Detection Buffer, pH7.4 (DB-05)** to prepare the Donor working solution. The working solution should be prepared immediately before use and must not be stored.

Note: Do not use the TR-FRET Sample Dilution Buffer, pH7.4 (DB-04), or any other buffers from external sources as substitutes, as doing so could result in unexpected or inconsistent experimental results.

3. Addition of Samples and Donor reagent

Add 10 µL of diluted samples and serially diluted standards to the designated wells according to our recommendation (Table 4) or your own plate layout. Then add 5 µL of the Donor working solution to each well. Seal the plate with a microplate sealing film and incubate at room temperature (20°C-25°C) for 30 minutes on an orbital shaker at 400-600 rpm to ensure the samples and Donor can react adequately.

4. Preparation of Acceptor working solution

Dilute the **FA-Labeled Human IL-21 R Protein (FRTP072-C02)** stock solution 50-fold with **TR-FRET Detection Buffer, pH7.4 (DB-05)** to prepare the Acceptor working solution. The working solution should be prepared immediately

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before use and must not be stored.

Note: The Detection Buffer of Acceptor is the same as Donor.

5. Addition of Acceptor reagent

Add 5 μL of Acceptor working solution to each well. Seal the plate with a microplate sealing film and incubate at room temperature (20℃-25℃) for 30 minutes on orbital shaker at 400-600 rpm.

Refer to Figure 4 and Table 4 for the recommended microplate layout. Add the corresponding reaction solutions to the designated wells according to the experimental requirements.

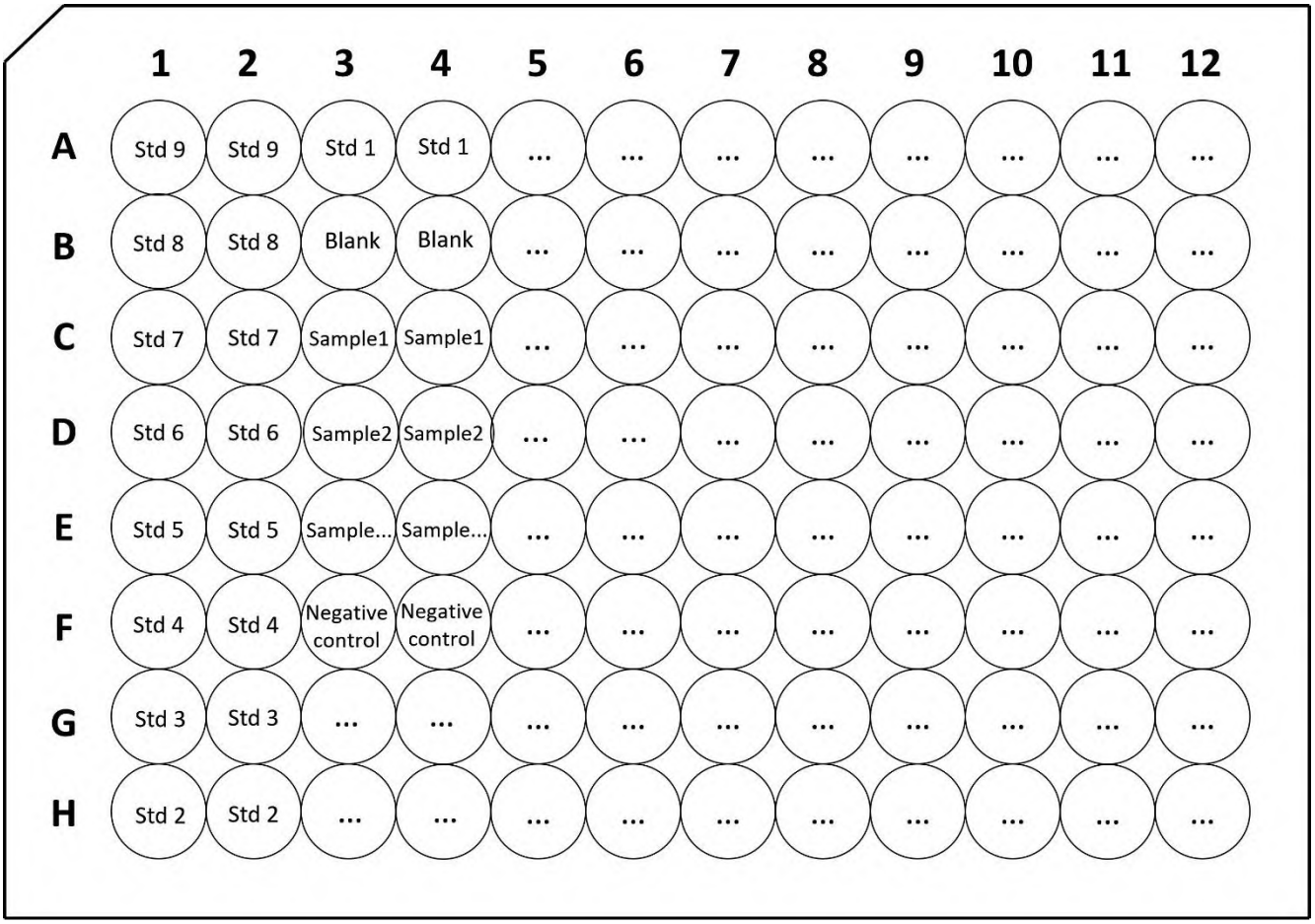
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TABLE 4. SAMPLES ADDING TO MICROPLATE

	1	2	3	4
A	10 μL Std 9 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Std 9 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Std 1 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Std 1 5 μL Donor working solution 5 μL Acceptor working solution
B	10 μL Std 8 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Std 8 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Sample Dilution Buffer 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Sample Dilution Buffer 5 μL Donor working solution 5 μL Acceptor working solution
C	10 μL Std 7 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Std 7 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Sample1 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Sample1 5 μL Donor working solution 5 μL Acceptor working solution
D	10 μL Std 6 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Std 6 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Sample2 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Sample2 5 μL Donor working solution 5 μL Acceptor working solution
E	10 μL Std 5 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Std 5 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Sample… 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Sample… 5 μL Donor working solution 5 μL Acceptor working solution
F	10 μL Std 4 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Std 4 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Sample Dilution Buffer 5 μL Donor working solution 5 μL Detection Buffer	10 μL Sample Dilution Buffer 5 μL Donor working solution 5 μL Detection Buffer
G	10 μL Std 3 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Std 3 5 μL Donor working solution 5 μL Acceptor working solution	…	…
H	10 μL Std 2 5 μL Donor working solution 5 μL Acceptor working solution	10 μL Std 2 5 μL Donor working solution 5 μL Acceptor working solution	…	…

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FIGURE 4. PLATE LAYOUT



6. Plate Reading

Use the TR-FRET module of a microplate reader to measure the fluorescence signals at 665 nm and 620 nm (the excitation wavelength is 337 nm).

7. Calculate Ratio

For each individual well, calculate the ratio of the Acceptor (665 nm) and Donor (620 nm) emission signals using the formula below:

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4.$$

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PRECAUTIONS

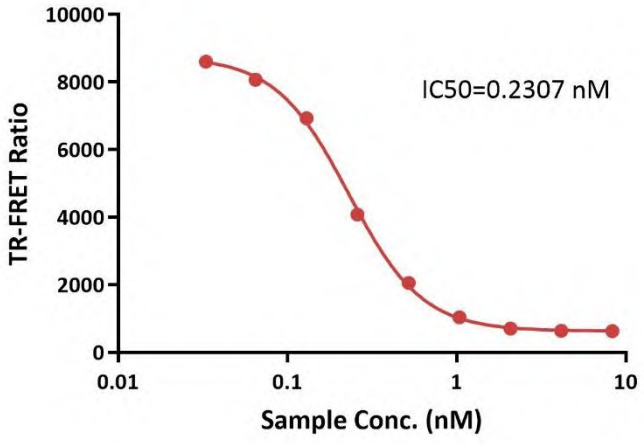
1. This kit is for research use only and is not intended for diagnostic or therapeutic applications.
2. Use this kit strictly according to the provided instructions.
3. Do not mix or substitute reagents with those from other kits or from kits of different lot numbers.
4. Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals form in any buffer solution, incubate the solution in a 37℃-40℃ water bath for 15-20 minutes until the crystals are completely dissolved. Before use, bring the solution back to room temperature.
5. Store this kit according to the storage instructions provided. Do not use reagents past their expiration date.
6. Prepare the working solution of each component according to the experimental requirements. All prepared working solutions are **for single use only** and **must not be stored.**
7. Ensure the correct use of dilution buffers for each component. Do not dilute samples with the Detection Buffer. Do not dilute Donor or Acceptor reagents with the Sample Dilution Buffer.

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TYPICAL DATA

For each experiment, a standard curve should be established for every microplate. The calculated Ratio values may vary depending on the laboratories, operator, and equipment. Different microplate readers and gain settings may produce different fluorescence signal intensities. Please adjust the instrument parameters according to the user manual. Reduce the gain value if the signal is too high. The following data were obtained using the BMG LABTECH PHERAstar® and are provided for reference only.

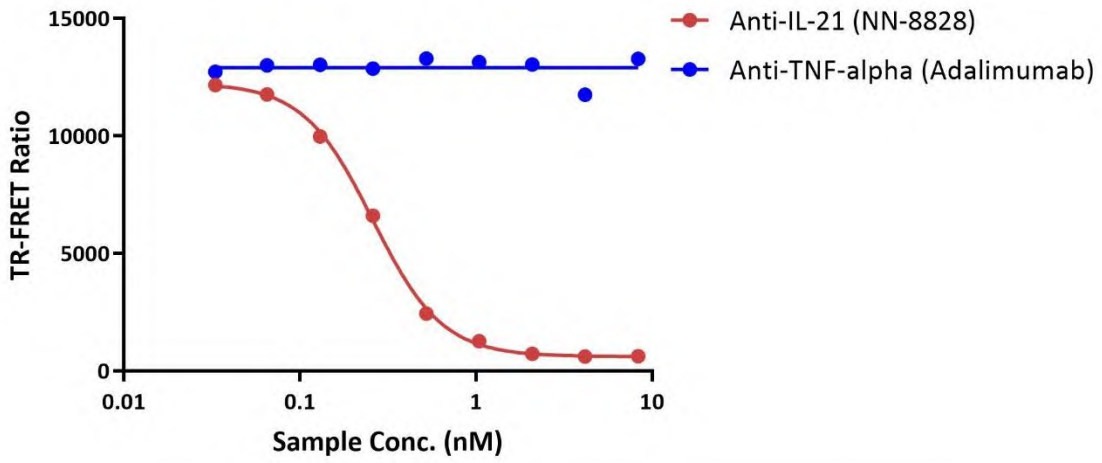
Anti-IL-21 Neutralizing Antibody		Ratio	SD	CV (%)
Conc. (μg/mL)	Conc. (nM)			
1.25	8.333	626	26	4.18
0.625	4.167	634	20	3.12
0.313	2.083	704	28	3.93
0.156	1.042	1037	25	2.38
0.078	0.521	2051	48	2.34
0.039	0.260	4076	187	4.58
0.020	0.130	6927	115	1.65
0.010	0.065	8067	87	1.08
0.005	0.033	8607	196	2.28
0	0	9056	59	0.65



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DIFFERENT ANTIBODY DATA

The kit is suitable for the detection and characterization of human IL-21 / IL-21R inhibitors. It was shown that the Anti-IL-21 (NN-8828) disrupted the interaction, with an IC_{50} of 0.2588 nM. The Anti-TNF-alpha (Adalimumab) antibody showed no significant inhibitory effect in the assay as expected. The following data were obtained using the BMG LABTECH PHERAstar® and are provided for reference only.



Sample	IC_{50} (nM)
Anti-IL-21 (NN-8828)	0.2588
Anti-TNF-alpha (Adalimumab)	-

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Large CV	* Inaccurate pipetting. * Air bubbles in wells.	* Check pipettes. * Remove bubbles in wells.
High background	* Reagent contamination. * Interfering components.	* Avoid contaminating the reagents. * Ensure the purity of the samples or dilute them to reduce interference.
Hook Effect	* Inappropriate sample detection concentration. * The usage concentration of the Donor/Acceptor is not applicable to certain special samples.	* Sample dilution optimization. * Optimize the usage concentration of Donor/Acceptor.
Very low readings across the plate	* Incorrect wavelengths or gain value set. * Insufficient reaction time.	* Check filters/gain/reader. * Increase reaction time.
Matrix interference	* Sample matrix does not match the standard curve.	* Dilute the sample using a higher dilution factor to reduce matrix interference. * Prepare the standard curve using the same matrix as the test sample to minimize matrix interference and obtain more accurate back-calculated sample concentrations.
Low instrument detection sensitivity	* Instrument parameters are not appropriately set.	* Set the instrument delay time to 50–100 μs and the integration time to 100–400 μs. * Adjust the instrument gain to an appropriate range to avoid signal oversaturation or excessively low signal levels.

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