

Description

The IL-21 (Human) Chemiluminescent ELISA Detection Kit is a sandwich ELISA designed for detecting and quantifying human interleukin-21 (IL-21) in cell culture medium. This kit comes with enough anti-IL-21 capture and detection antibodies, IL-21 standard, and detection reagents for 100 enzyme reactions.

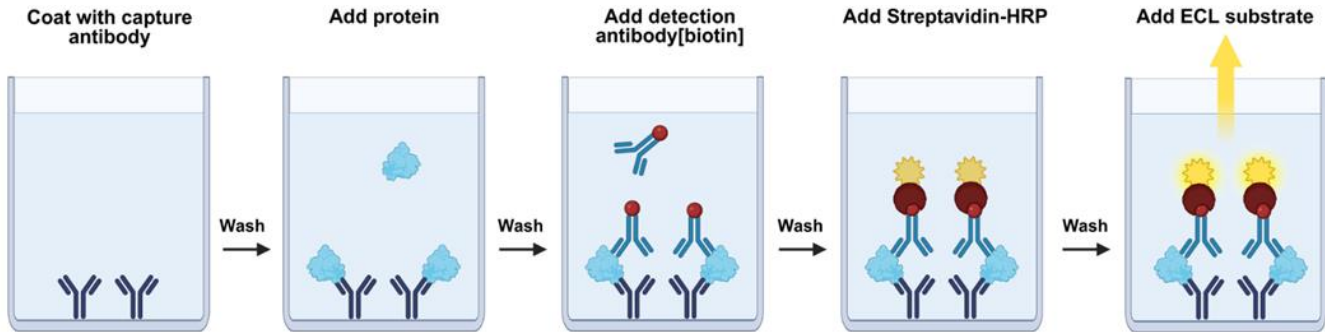


Figure 1. IL-21 (Human) Chemiluminescent ELISA Detection Kit schematic.

A 96-well plate is coated with an anti-IL-21 capture antibody. After coating and blocking, samples containing IL-21 are added. Next, unbound IL-21 is washed away, and the plate is incubated with biotinylated IL-21 detection antibody. After another wash, streptavidin-HRP is added. After a final wash, ELISA ECL substrate is added to produce chemiluminescence that can be measured using a chemiluminescence reader. The chemiluminescence signal is proportional to the amount of IL-21 in the samples.

Background

IL-21 (interleukin-21) is a cytokine mainly produced by CD4⁺ T cells that triggers effector function and memory differentiation of CD8⁺ T cells during infection and in cancer (such as Hodgkin's lymphoma). IL-21 expression is also found in NKT cells, where it regulates their function. It binds to the receptor IL-21R, found on the surface of T, B and NK cells. In B cells it leads to the generation of high-affinity class-switched antibodies. The functional signaling complex activates Janus kinases JAK1, JAK3, and the STAT (signal transducer and activator of transcription) proteins STAT1 and STAT3, impacting cell survival and proliferation. High levels of IL-21, expression of IL21R and polymorphisms in both IL-21 and IL21R have been described in autoimmune diseases. The development of therapeutic strategies targeting IL-21 will impact patients with SLE (systemic lupus erythematosus), type I diabetes, MS (multiple sclerosis), IBD (inflammatory bowel disease), and psoriasis.

Applications

Quantify IL-21 in cell culture medium.

Supplied Materials

Catalog #	Name	Amount	Storage
83696-KC10 ⁺	IL-21 Capture Antibody*	10 µg	-80°C
83697-KC6 ⁺	IL-21 Detection Antibody, Biotinylated*	6 µl	-80°C
83698-KC7.5 ⁺	IL-21 Standard*	>7.5 ng	-80°C
79743	Blocking Buffer 3	50 ml	+4°C
82724-KC6	Streptavidin HRP	6 µl	+4°C
79670-KC6	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
79699	White 96-well microplate	1	Room Temp

*The concentration of the protein is lot-specific and will be indicated on the tube.

Materials Required but Not Supplied

- 1x PBS (Phosphate Buffer Saline) Buffer
- PBST Buffer (1x PBS, containing 0.05% Tween-20)
- Luminometer or microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

This kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples and controls should be performed in duplicate.
- We recommend using IL-21 Standard (#83698-KC7.5⁺) as “Standard” and generating a standard curve for each experiment.
- The assay should include “Blank”, “Standard”, and “Test Sample” conditions.
- Variation in sample collection, processing and storage may cause differences in sample values.

Step 1: Coat 96-well plate

Coat the plate one day prior to running your samples.

1. Thaw **IL-21 Capture Antibody** on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute **IL-21 Capture Antibody** to 2 ng/ μ l with 1x PBS (50 μ l/well).
3. Add 50 μ l of **diluted IL-21 Capture Antibody** to every well.
4. Incubate at 4°C overnight.
5. Wash the plate three times using 200 μ l of **PBST Buffer** per well.
6. Tap the plate onto clean paper towels to remove the liquid.
7. Block the wells by adding 200 μ l of **Blocking Buffer 3** to every well.
8. Incubate at Room Temperature (RT) for at least 90 minutes.
9. Wash the plate three times using 200 μ l of **PBST Buffer** per well.
10. Tap the plate onto clean paper towels to remove the liquid.

Step 2: Quantification

1. Briefly spin the tube of **IL-21 Standard** to recover the full content of the tube. Resuspend the **IL-21 Standard** at RT in 6 μ l of distilled water for a concentration of 10 μ g/ml.
2. Dilute the IL-21 Standard to 1 μ g/ml with Blocking Buffer 3.
3. Prepare a serial dilution of the **IL-21 Standard** (50 μ l/well), starting at 25,000 pg/ml, as described in the table below using the same diluent as the test sample, for example, the same culture medium.

Note: A linear response is seen between 25,000 pg/ml to 780 pg/ml if Blocking Buffer 3 is used as diluent.

Dilution Series	Volume of IL-21 Standard stock or previous dilution (µl)	Volume of Diluent (µl)	pg/ml
Dilution 1	7.5 µl of IL-21 Standard stock (1 µg/ml)	292.5 µl	25000
Dilution 2	150 µl of Dilution 1	150 µl	12500
Dilution 3	150 µl of Dilution 2	150 µl	6250
Dilution 4	150 µl of Dilution 3	150 µl	3125
Continue Dilutions As Above (for a total of 6 dilutions + blank)			
Blank	-	150 µl	0

4. Add 50 µl of serially **diluted IL-21 Standard** to the “Standard” wells.
5. Prepare the **test sample**. If dilutions are necessary, use Blocking Buffer 3 (50 µl/well).
6. Add 50 µl of the **test sample** to the “Test Sample” wells.
7. Add 50 µl of the diluent to “Blank” wells.

Note: Use the same diluent as used in the test sample, for example, the same culture medium.

	Blank	Standard	Test Sample
Diluent	50 µl	-	-
Test Sample	-	-	50 µl
IL-21 Standard	-	50 µl	-
Total	50 µl	50 µl	50 µl

8. Incubate for 2 hours at RT with slow agitation.
9. Wash the plate three times with 200 µl of **PBST Buffer** per well and tap the plate onto clean paper towels.

Step 3: Detection

1. Thaw **IL-21 Detection Antibody** on ice.
2. Dilute **IL-21 Detection Antibody** 1000-fold with Blocking Buffer 3 (50 µl/well).
3. Add 50 µl of **diluted IL-21 Detection Antibody** to every well.
4. Incubate for 1 hour at RT.
5. Wash the plate three times with 200 µl of **PBST Buffer** per well and tap the plate onto clean paper towels.

6. Dilute **Streptavidin-HRP** 1000-fold with Blocking Buffer 3 (50 µl/well).
7. Add 50 µl of **diluted Streptavidin-HRP** to every well.
8. Incubate for 30 minutes at RT.
9. Wash the plate three times with 200 µl of **PBST Buffer** per well and tap the plate onto clean paper towels.
10. Just before use, mix 1 volume of **ELISA ECL Substrate A** and 1 volume of **ELISA ECL Substrate B** (100 µl of mix/well).
11. Add 100 µl of mix to every well.
12. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.
13. The “Blank” value should be subtracted from all other values.

Reading Chemiluminescence

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the “hole” position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of controls.

Example Results

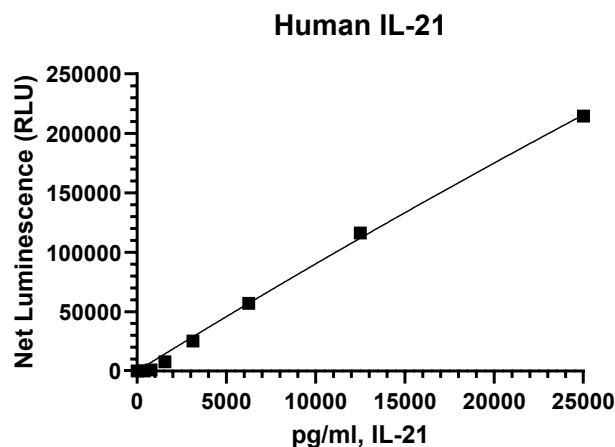


Figure 2: IL-21 Standard titration curve.

Various amounts of IL-21 Standard prepared in Blocking Buffer 3 were run in duplicate. A linear response is seen between 25,000 pg/ml to 780 pg/ml.

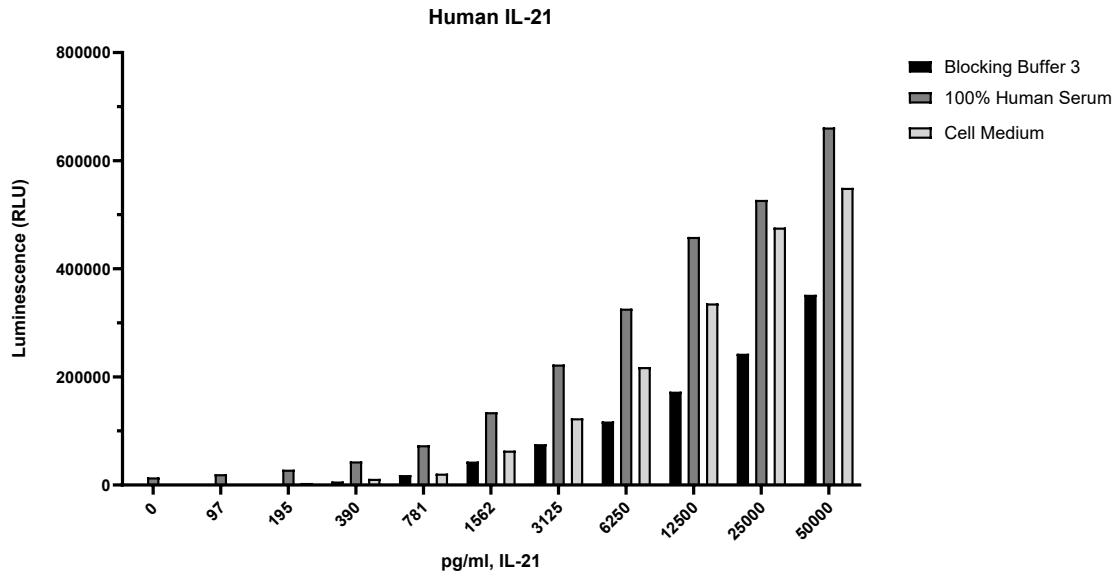


Figure 3: IL-21 Standard titration curve in various diluents. Detection of IL-21 Standard in different solutions: Blocking Buffer 3 (#79743), 100% Human Serum (Sigma-Aldrich #P2918), and Cell Medium (DMEM supplemented with 10% FBS).

Data shown is representative.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit <https://bpsbioscience.com/contact>.

References

Spolski R. and Leonard W., 2014 *Nature Reviews Drug Discovery* 13: 379-395.

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