Description

The IL-21 (Human) Colorimetric ELISA Detection Kit is a sandwich ELISA assay 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 5 x 100 enzyme reactions.

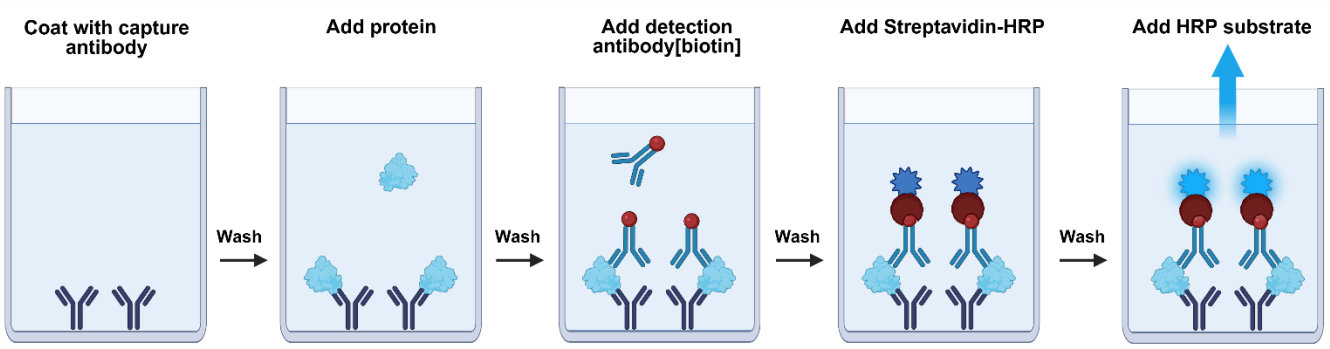

Figure 1. IL-21 (Human) Colorimetric ELISA Detection Kit schematic.
A 96-well plate is coated with an anti-IL-21 capture antibody. After coating and blocking, samples containing IL-21 are added. Next, unbound IL-21 is washed away, and the plate is incubated with biotinylated IL-21 detection antibody. After another wash, streptavidin-HRP is added. After a final wash, HRP Colorimetric Substrate is added to produce absorbance that can be measured using a UV/Vis spectrophotometer microplate reader. The absorbance 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 Iin 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*	5 x 10 µg	-80°C
83697-KC6^{+}	IL-21 Detection Antibody, Biotinylated*	5 x 6 µl	-80°C
83698-KC2.5^{+}	IL-21 Standard*	5 x >2.5 ng	-80°C
79743	Blocking Buffer 3	5 x 50 ml	+4°C
82724-KC6	Streptavidin HRP	5 x 6 µl	+4°C
79651	HRP Colorimetric Substrate	5 x 10 ml	+4°C
79964	Transparent 96-well plate	5 x 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)
- 2 M sulfuric acid
- UV/Vis spectrophotometer microplate reader capable of reading absorbance
- 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-KC2.5^{+}) 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 antibody to recover its full content.
2. Dilute **IL-21 Capture Antibody** to 2 ng/µl with 1x PBS (50 µl/well).
3. Add 50 µl of **diluted IL-21 Capture Antibody** to every well.
4. Incubate at 4°C overnight.
5. Wash the plate three times using 200 µl of **PBST Buffer** per well.
6. Tap the plate onto clean paper towels to remove the liquid.
7. Block the wells by adding 200 µl 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 µl 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 µl of distilled water for a concentration of 10 µg/ml.
2. Dilute the IL-21 Standard to 1 µg/ml with Blocking Buffer 3.
3. Prepare a serial dilution of the **IL-21 Standard** (50 µl/well), starting at 6,250 pg/ml, as described in the table below using the same diluent as in the test sample, for example, the same culture medium.

   Note: A linear response is seen between 6,250 pg/ml to 390 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	3.75 µl of IL-21 Standard stock (1 µg/ml)	596.25 µl	6250
Dilution 2	150 µl of Dilution 1	150 µl	3125
Dilution 3	150 µl of Dilution 2	150 µl	1562.5
Dilution 4	150 µl of Dilution 3	150 µl	781.25
Continue Dilutions As Above (for a total of 5 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. Add 100 µl of the **HRP Colorimetric Substrate** to each well.

11. Incubate the plate at RT until blue color is developed in the highest concentration of the "Standard" wells.

    Note: It normally takes 10-15 minutes to fully develop the color. However, the optimal incubation time may vary and should be determined empirically by the user. If color is intense the plate can be read right away at 650 nm without adding 2 M sulfuric acid (see below). To increase the Signal-to-Background ratio proceed to the next step.

12. Add 100 µl of **2 M sulfuric acid** to each well.

13. Read the absorbance at 450 nm using a UV/Vis spectrophotometer microplate reader.

14. The "Blank" value should be subtracted from all other values.

Example Results

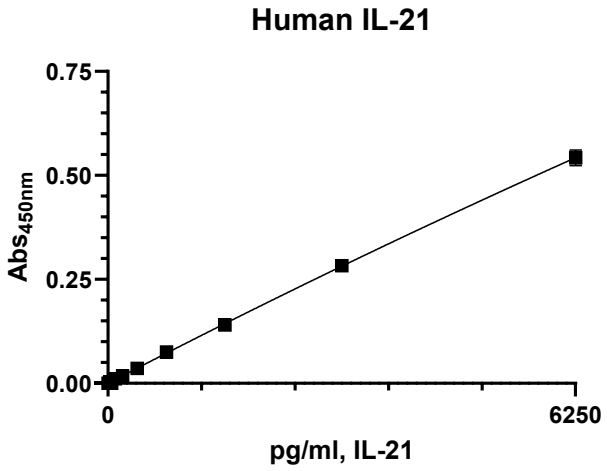

Figure 2: IL-21 Standard titration curve.
Various amounts of the IL-21 Standard prepared in Blocking Buffer 3 were run in duplicate. A linear response is seen between 6,250 pg/ml to 390 pg/ml.

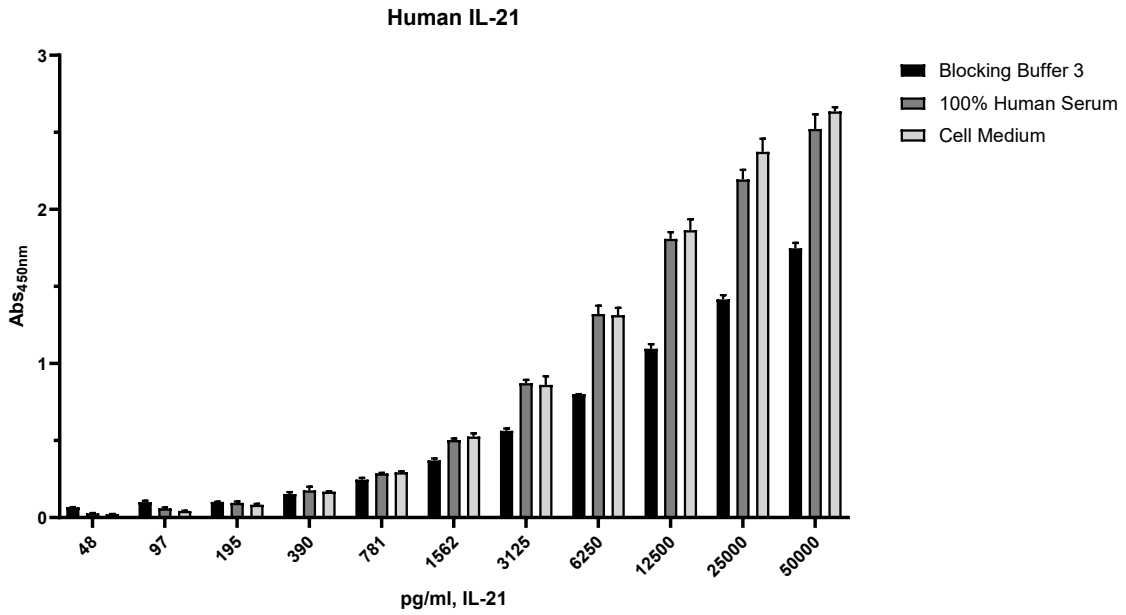

Figure 3: IL-21 Standard titration curve in various solutions.
Net absorbance for IL-21 Standard in different solutions: Blocking Buffer 3 (#79743), 100% Human Serum (Sigma-Aldrich #P2918), and Cell Medium (DMEM supplemented with 10% FBS).

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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