Description

The IL-5 (Human) Colorimetric ELISA Detection Kit is a sandwich ELISA designed for detecting and quantifying human interleukin-5 (IL-5) in cell culture medium. This kit comes with enough anti-IL-5 capture and detection antibodies, IL-5 standard, and detection reagents for 100 enzyme reactions.

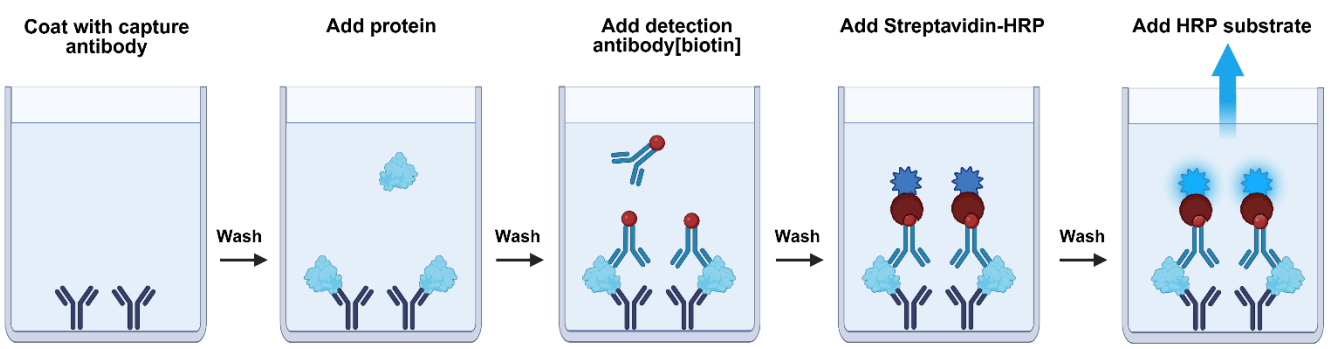

Figure 1. IL-5 (Human) Colorimetric ELISA Detection Kit schematic.

A 96-well plate is coated with an anti-IL-5 capture antibody. After coating and blocking, samples containing IL-5 are added. Next, unbound IL-5 is washed away, and the plate is incubated with the biotinylated IL-5 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-5 in the samples.

Background

IL-5 (interleukin-5) is a pro-inflammatory protein that functions as an intermediary cytokine in the T_H2 (T helper 2) cells response in asthma and other eosinophilic allergic diseases. IL-5 is produced by T_H2 cells and ILC2 (group 2 innate lymphoid cells) cells in response to stimulation by alarmins such as TSLP (thymic stromal lymphopoietin) or IL-33, which initiate the inflammatory cascade. IL-5 functions by promoting eosinophil maturation, activation, and migration into target tissues. It binds to the IL-5 specific cell surface receptor IL-5RA (IL-5 receptor alpha) and further recruits the common beta chain receptor (CSF2RB) to initiate downstream signaling through JAK1/2 (Janus kinase 1/2) and STAT5. The detection of IL-5 allows assessment of the activation of T helper cells.

Applications

Quantify IL-5 in cell culture medium.

Supplied Materials

Catalog #	Name	Amount	Storage
83691-KC10^{+}	IL-5 Capture Antibody*	10 µg	-80°C
83692-KC6^{+}	IL-5 Detection Antibody, Biotinylated*	6 µl	-80°C
102506-KC15	IL-5 Standard*	15 µl	-80°C
79743	Blocking Buffer 3	50 ml	+4°C
82724-KC6	Streptavidin HRP	6 µl	+4°C
79651	HRP Colorimetric Substrate	10 ml	+4°C
79964	Transparent 96-well plate	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-5 Standard (#102506-KC15) 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-5 Capture Antibody** on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute **IL-5 Capture Antibody** to 2 ng/µl with 1x PBS (50 µl/well).
3. Add 50 µl of **diluted IL-5 Capture Antibod**y 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 towel 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. Thaw the **IL-5 Standard** on ice. Briefly spin the tube to recover the full content of the tube.
2. Prepare a serial dilution of the **IL-5 Standard** (50 µl/well), starting at 20,000 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 20,000 pg/ml to 300 pg/ml if Blocking Buffer 3 is used as a diluent.

Dilution Series	Volume of IL-5 Standard stock or previous dilution (µl)	Volume of Diluent (µl)	pg/ml
Dilution 1	6 of IL-5 Standard stock	294 µl	20000
Dilution 2	150 µl of Dilution 1	150 µl	10000
Dilution 3	150 µl of Dilution 2	150 µl	5000
Dilution 4	150 µl of Dilution 3	150 µl	2500
Continue Dilutions As Above (for a total of 7 dilutions + blank)			
Blank	-	150 µl	0

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

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

	Blank	**Standard**	**Test Sample**
Diluent	50 µl	-	-
Test Sample	-	-	50 µl
IL-5 Standard	-	50 µl	-
Total	**50 µl**	**50 µl**	**50 µl**

7. Incubate for 2 hours at RT with slow agitation.
8. 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-5 Detection Antibody** on ice.
2. Dilute **IL-5 Detection Antibody** 1000-fold with Blocking Buffer 3 (50 µl/well).
3. Add 50 µl of **diluted IL-5 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

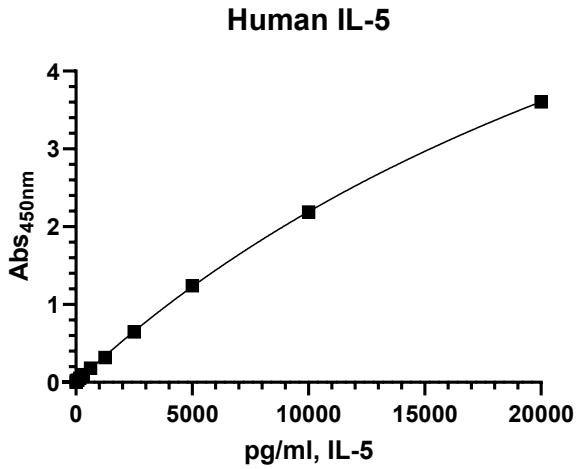

Figure 2: IL-5 standard titration curve.
Various amounts of the IL-5 Standard prepared in Blocking Buffer 3 were run in duplicate. A linear response is seen between 20,000 pg/ml to 300 pg/ml.

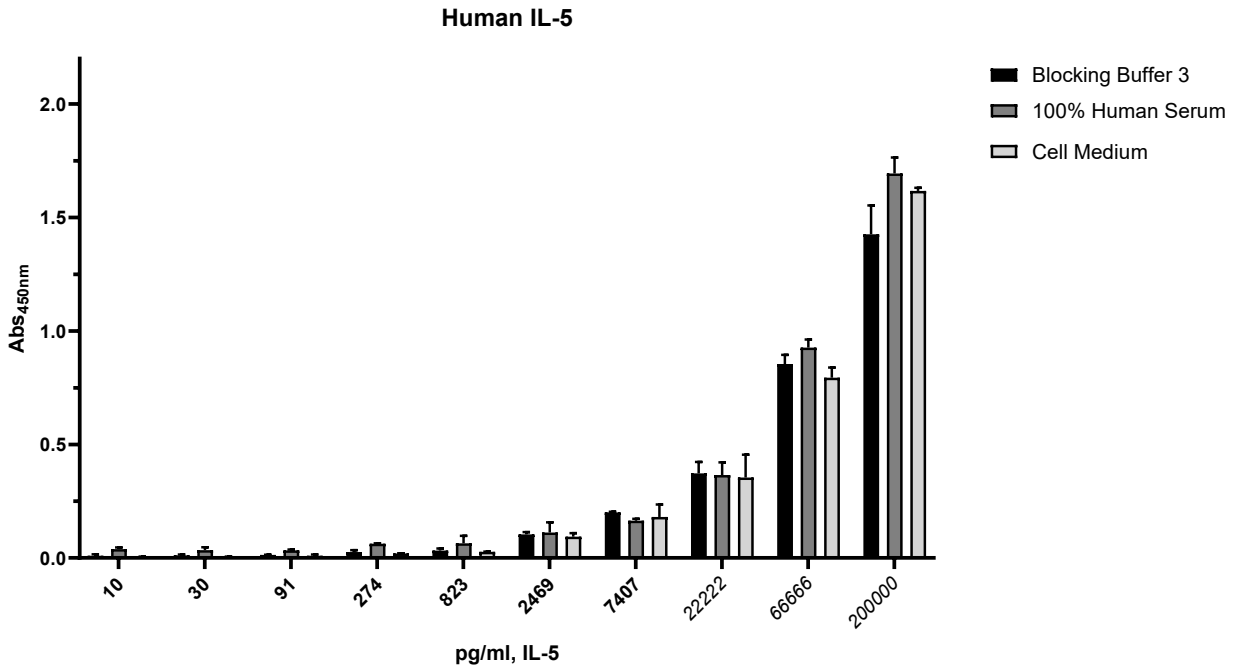

Figure 3: IL-5 standard titration curve in various solutions.
Net absorbance for IL-5 Standard in different solutions: Blocking Buffer 3 (#79743), 100% Human Serum (Sigma-Aldrich #P2918), and Cell Medium (DMEM supplemented with 10% FBS).

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

Schleich F.*, et al.,* 2023 *Eur Respir Rev* 32(168): 220193.

Related Products

Products	*Catalog #*	*Size*
IL-4 (Human) Chemiluminescence ELISA Detection Kit	83633	96 reactions/ 5 x 96 reactions
IL-4 (Human) Colorimetric ELISA Detection Kit	83780	96 reactions/ 5 x 96 reactions
IL-5 (Human) Chemiluminescence ELISA Detection Kit	83634	96 reactions/ 5 x 96 reactions
IL-13 (Human) Chemiluminescence ELISA Detection Kit	83636	96 reactions/ 5 x 96 reactions
IL-2 (Human) Colorimetric ELISA Detection Kit	79774	96 reactions/ 5 x 96 reactions

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