Description

The IL-13 (Human) Chemiluminescent ELISA Detection Kit is a sandwich ELISA assay designed for detecting and quantifying human interleukin-13 (IL-13) in cell culture medium. This kit comes with enough anti-IL-13 capture and detection antibodies, IL-13 standard, and detection reagents for 100 enzyme reactions.

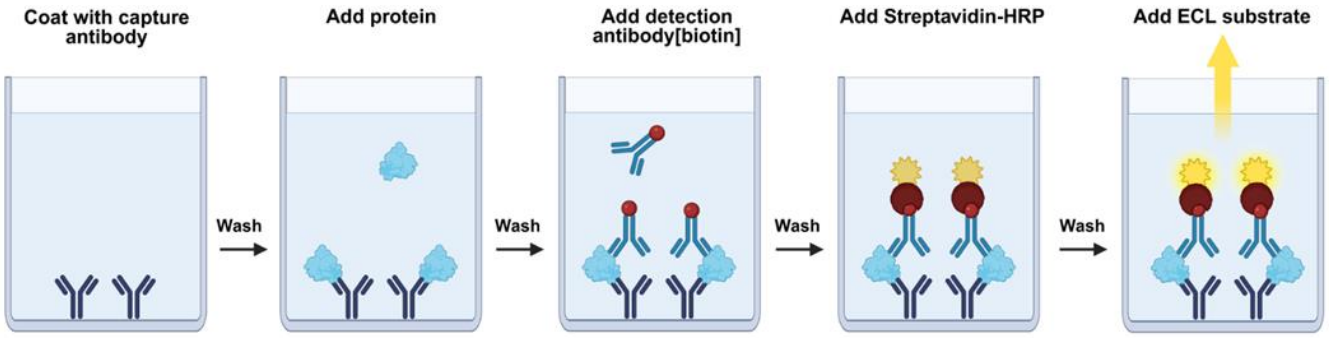

Figure 1. IL-13 (Human) Chemiluminescent ELISA Detection Kit schematic.
A 96-well plate is coated with an anti-IL-13 capture antibody. After coating and blocking, samples containing IL-13 are added. Next, unbound IL-13 is washed away, and the plate is incubated with biotinylated IL-13 detection antibody. 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-13 in the samples.

Background

The transcription factor STAT6 (signal transduced and activator of transcription factor 6) plays a vitally important role in immune cells. STAT6 is activated primarily by IL-4 and the related IL-13, with have overlapping biologic profiles. Upon IL-4 and IL-13 binding, the receptor complex composed of IL-4Ralpha and IL-13Ralpha1 activates the receptor-associated Janus kinases (JAK1 and Tyk2), leading to the activation of STAT6. Activated STAT6 forms homodimers that translocate to the nucleus where they bind the promoter of responsive genes, inducing gene transcription. IL-13 is a cytokine secreted by many cell types, but especially T helper type 2 (Th2) cells, and is an important mediator of allergic inflammation and disease. The detection of IL-13 allows assessment of the activation of T helper cells.

Applications

Quantification of IL-13 in cell culture medium.

Supplied Materials

Catalog #	Name	Amount	Storage
83712-KC10^{+}	IL-13 Capture Antibody*	10 µg	-80°C
83713-KC6^{+}	IL-13 Detection Antibody, Biotinylated*	6 µl	-80°C
83714-KC2.4^{+}	IL-13 Standard*	>2.4 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-13 Standard (#83714-KC2.4^{+}) 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-13 Capture Antibody** on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute **IL-13 Capture Antibody** to 2 ng/µl with 1x PBS (50 µl/well).
3. Add 50 µl of **diluted IL-13 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 to recover the full content of the tube. Resuspend the **IL-13 Standard** at RT in 8 µl of distilled water for a concentration of 0.5 µg/ml.
2. Prepare a serial dilution of the **IL-13 Standard** (50 µl/well), starting at 8,000 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 8,000 pg/ml to 50 pg/ml if Blocking Buffer 3 is used as a diluent.

Dilution Series	Volume of IL-13 Standard stock or previous dilution (µl)	Volume of Diluent (µl)	pg/ml
Dilution 1	4.8 µl of IL-13 Standard stock	295.2 µl	8000
Dilution 2	150 µl of Dilution 1	150 µl	4000
Dilution 3	150 µl of Dilution 2	150 µl	2000
Dilution 4	150 µl of Dilution 3	150 µl	1000
Continue dilutions as above (for a total of 8 dilutions + blank)			
Blank	-	150 µl	0

3. Add 50 µl of serially **diluted IL-13 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** to the "Test Sample" wells.
6. Add 50 µl of the diluent to "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-13 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-13 Detection Antibody** on ice.
2. Dilute **IL-13 Detection Antibody** 1000-fold with Blocking Buffer 3 (50 µl/well).
3. Add 50 µl of **diluted IL-13 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

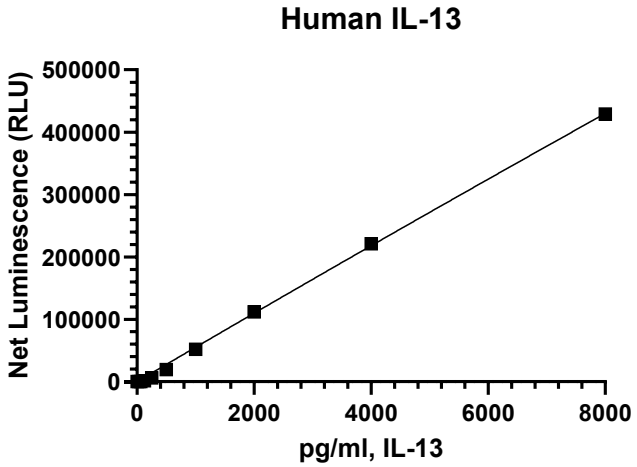

Figure 2: IL-13 standard titration curve.
Various amounts of the IL-13 Standard prepared in Blocking Buffer 3 were run in duplicate. A linear response is seen between 8,000 pg/ml to 50 pg/ml.

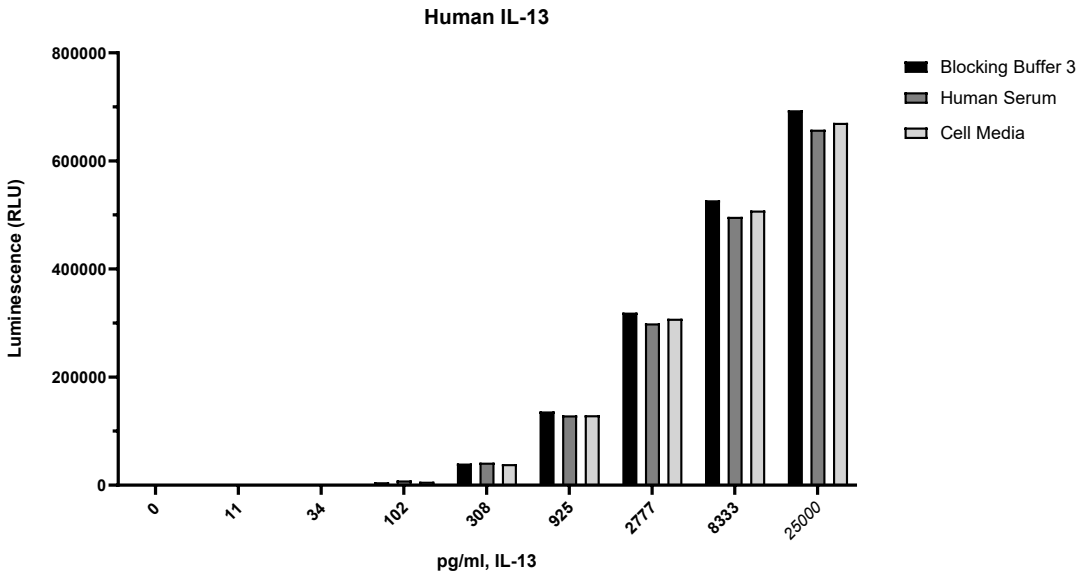

Figure 3: IL-13 standard titration curve in various solutions.
Detection of IL-13 p40 Standard in different solutions: Blocking Buffer 3 (#79743), 100% Human Serum (Sigma-Aldrich #P2918), and Cell Medium (DMEM supplemented with 10% FBS).

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

Kasaian MT., *et al.*, 2013. *Am J Respir Cell Mol Biol.* 49(1):37-46.
Czimmerer Z., *et al.*, 2018. *Immunity* 48(1):75-90.e6.

Related Products

Products	*Catalog #*	*Size*
IL-4 (Human) Chemiluminescence ELISA Detection Kit	83633	96 reactions/ 5 x 96 reactions
IL-5 (Human) Colorimetric ELISA Detection Kit	83781	96 reactions/ 5 x 96 reactions
IL-5 (Human) Chemiluminescence ELISA Detection Kit	83634	96 reactions/ 5 x 96 reactions
IL-13 (Human) Colorimetric ELISA Detection Kit	83783	96 reactions/ 5 x 96 reactions
IL-2 (Human) Colorimetric ELISA Detection Kit	79774	96 reactions/ 5 x 96 reactions

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