

# Rabbit IL-8 (CXCL8) ELISA Kit

Cat. No. E121-800

## Components Supplied

- **Rabbit IL-8 Pre-Coated 96-well Strip Plate**, 1 each
- **Rabbit IL-8 Standard**, 640 pg/vial, 2 each
- **Rabbit IL-8 Detection Antibody**, 12 ml
- **Dilution Buffer A**, 25 ml
- **HRP Solution**, 12 ml
- **TMB Substrate**, 12 ml
- **Stop Solution**, 12 ml
- **20X Wash Buffer**, 50 ml
- **Sealing Tape**, 6 sheets

## Introduction

Enzyme linked immunosorbent assay (ELISA) for the detection of Rabbit IL-8 (CXCL8) in cell culture supernatants. Kit contains sufficient components to quantitate Rabbit IL-8 protein concentration in up to 40 samples, tested in duplicate.

**Bethyl Laboratories, Inc. • [www.bethyl.com](http://www.bethyl.com)**  
**Telephone: 800-338-9579 • Fax: 866-597-6105**

## **Background**

Interleukin-8 (IL-8), renamed CXCL8, is a chemokine produced by macrophages and other cell types such as epithelial cells and endothelial cells. IL-8 can be secreted by any cell with toll-like receptors which are involved in the innate immune response. The most frequently studied receptors of IL-8 are the G protein coupled serpentine receptors CXCR1 (previously named IL-8 receptor  $\alpha$ ) and CXCR2 (previously named IL-8 receptor  $\beta$ ). Both monomer and homodimer forms of IL-8 were reported as potent inducers of CXCR1 and CXCR2.

IL-8 is one of the first major mediators of the inflammatory response. The primary function of IL-8 is the induction of chemotaxis in its target cells (e.g. neutrophil granulocytes). Initially, macrophages phagocytose the antigen. Upon processing the antigen, macrophages release chemokines, such as IL-8, to signal other immune cells to come to the site of inflammation. IL-8 serves as a chemical signal that attracts neutrophils at the site of inflammation, and therefore is also known as *neutrophil chemotactic factor*.

While neutrophil granulocytes are the primary target cells of IL-8, there is a relative wide range of cells (endothelial cells, macrophages, mast cells, keratinocytes) responding to this chemokine, too. IL-8 is believed to play a role in the pathogenesis of bronchiolitis, a common respiratory tract disease caused by viral infection.

**Bethyl Laboratories, Inc. • [www.bethyl.com](http://www.bethyl.com)  
Telephone: 800-338-9579 • Fax: 866-597-6105**

## **Procedure Overview**

1. Add 100  $\mu$ l of standard or sample to well.  
Note: Run each standard or sample in duplicate.
- ↓
2. Cover plate and incubate at room temperature (20-25°C) for 1 hour.
- ↓
3. Wash plate FOUR times.
- ↓
4. Add 100  $\mu$ l of Rabbit IL-8 Detection Antibody to each well.
- ↓
5. Cover plate and incubate at room temperature for 1 hour.
- ↓
6. Wash plate FOUR times.
- ↓
7. Add 100  $\mu$ l of HRP Solution to each well.
- ↓
8. Cover plate and incubate at room temperature for 30 minutes.
- ↓
9. Wash plate FOUR times.
- ↓
10. Add 100  $\mu$ l of TMB Substrate Solution to each well.
- ↓
11. Develop the plate in the dark at room temperature for 30 minutes.
- ↓
12. Stop reaction by adding 100  $\mu$ l of Stop Solution to each well.
- ↓
13. Measure absorbance on a plate reader at 450 nm.

**Bethyl Laboratories, Inc. • [www.bethyl.com](http://www.bethyl.com)  
Telephone: 800-338-9579 • Fax: 866-597-6105**

### **Additional Materials Required**

- Ultrapure water
- Precision pipettors, with disposable plastic tips
- Polypropylene or polyethylene tubes to prepare standard and samples – do not use polystyrene, polycarbonate or glass tubes
- A container to prepare 1X Wash Buffer
- A wash bottle or an automated 96-well plate washer
- Disposable reagent reservoirs
- A standard microtiter plate reader for measuring absorbance at 450 nm

### **Precautions**

- Store all reagents at 2-8°C. Do not freeze reagents.
- All reagents must be at room temperature (20-25°C) before use.
- Vigorous plate washing is essential.
- Use new disposable pipette tips for each transfer to avoid cross-contamination.
- Use a new adhesive plate cover for each incubation step.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Avoid microbial contamination of reagents and equipment. Automated plate washers can easily become contaminated thereby causing assay variability.
- Take care not to contaminate the TMB Solution. Do not expose TMB Substrate solution to glass, foil, or metal. If the solution is blue before use, DO NOT USE IT.
- Individual components may contain preservatives. Wear gloves while performing the assay. Please follow proper disposal procedures.

**Bethyl Laboratories, Inc. • [www.bethyl.com](http://www.bethyl.com)  
Telephone: 800-338-9579 • Fax: 866-597-6105**

## **Standard and Sample Handling and Preparation**

### **Standard Preparation**

1. Reconstitute 640 pg standard vial with 1 ml of Dilution Buffer A to achieve a concentration of 640 pg/ml. Mix well. Dilute 500  $\mu$ l of reconstituted standard in 500  $\mu$ l Dilution Buffer A. Mix well. This is the top standard with a final concentration of 320 pg/ml.
2. Label seven (7) tubes, one for each additional standard curve point: 160 pg/ml, 80 pg/ml, 40 pg/ml, 20 pg/ml, 10 pg/ml, 5 pg/ml, and 0 pg/ml.
3. Pipette 250  $\mu$ l of Dilution Buffer A into tubes.
4. Serial dilute the 320 pg/ml standard 1:1 with Dilution Buffer A. Perform dilution by mixing 250  $\mu$ l of the previous standard with 250  $\mu$ l of Dilution Buffer A. Continue until reach standard value of 5 pg/ml.
5. Use Dilution Buffer A only as the zero standard value.

### **Sample Handling**

- Cell culture supernatants may be tested in this ELISA.
- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing of infectious agents.
- 100  $\mu$ l of sample or standard is required per well.
- Samples must be assayed in duplicate each time the assay is performed.
- Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -70°C. Avoid repeated freeze-thaw cycles when storing samples.
- If particulate is present in samples, centrifuge prior to analysis.
- If the integrity of the sample is of concern, make a note on the Plate Template and interpret results with caution.

### **Sample Preparation**

- If it is suspected that the concentration of the sample exceeds the highest point of the standard curve, prepare one or more dilutions of the sample in Dilution Buffer A until the desired concentration is obtained. For example,

**Bethyl Laboratories, Inc. • [www.bethyl.com](http://www.bethyl.com)  
Telephone: 800-338-9579 • Fax: 866-597-6105**

a 1:10 dilution could be prepared by diluting 25  $\mu$ l of sample in 225  $\mu$ l of Dilution Buffer A in a clean, fresh tube and mixing well.

### **1X Wash Buffer Preparation**

- Prepare 1X Wash Buffer by diluting 20X Wash Buffer in ultra pure water. For example, if preparing 1 L of 1X Wash Buffer, dilute 50 ml of 20X Wash Buffer into 950 ml of ultrapure water. Mix well. Store reconstituted 1X Wash Buffer at 2-8°C for up to six (6) months. Do not use 1X Wash Buffer if it becomes visibly contaminated during storage.

## **Assay Procedure**

### **Sample Incubation**

- Determine the number of strips required. Leave these strips in the plate frame. Place unused strips in the foil pouch with desiccant and seal tightly. Store unused strips at 2-8°C. After completing assay, keep the plate frame for additional assays.
  - Use a Plate Template to record the locations of the standards and unknown samples within the wells.
1. Add 100  $\mu$ l of appropriately diluted standards or samples to each well. Run each standard, sample, or blank in duplicate.
  2. Carefully cover wells with a new adhesive plate cover. Incubate for one (1) hour at room temperature, 20-25°C.
  3. Carefully remove adhesive plate cover, discard plate contents and wash FOUR times with 1X Wash Buffer as described in the Plate Washing section.

### **Plate Washing**

1. Gently squeeze the long sides of plate frame before washing to ensure all strips remain securely in the frame.
2. Empty plate contents. Use a squirt wash bottle to vigorously fill each well completely with 1X Wash Buffer, then empty plate contents. Repeat procedure three additional times for a total of FOUR washes. Blot plate onto paper towels or other absorbent material.

**Bethyl Laboratories, Inc. • [www.bethyl.com](http://www.bethyl.com)  
Telephone: 800-338-9579 • Fax: 866-597-6105**

**Note:** For automated washing, aspirate plate contents from all wells and flood wells with 1X Wash Buffer. Repeat procedure three additional times for a total of FOUR washes. Additional washes may be necessary. Blot plate onto paper towels or other absorbent material.

Take care to avoid microbial contamination of equipment. Automated plate washers can easily become contaminated thereby causing assay variability.

#### **Detection Antibody Incubation**

- Only remove the required amount of Detection Antibody reagent for the number of strips being used.
1. Add 100 µl of Detection Antibody to each well containing standard, sample or blank. Mix well by gently tapping the plate several times.
  2. Carefully attach a new adhesive plate cover. Incubate plate for one (1) hour at room temperature, 20-25°C.
  3. Carefully remove the adhesive plate cover, discard plate contents and wash FOUR times with 1X Wash Buffer as described in the Plate Washing section.

#### **HRP Solution Incubation**

- Only remove the required amount of HRP Solution for the number of strips being used.
1. Add 100 µl of HRP Solution to each well containing sample or blank.
  2. Carefully attach a new adhesive plate cover. Incubate plate for 30 minutes at room temperature, 20-25°C.
  3. Carefully remove the adhesive plate cover, discard plate contents and wash FOUR times with 1X Wash Buffer as described in the Plate Washing section.

#### **TMB Substrate Incubation and Reaction Stop**

- Only remove the required amount of TMB Substrate Solution and Stop Solution for the number of strips being used.
- Do NOT use a glass pipette to measure the TMB Substrate Solution. Do NOT cover the plate with aluminum foil or metalized mylar. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused

**Bethyl Laboratories, Inc. • [www.bethyl.com](http://www.bethyl.com)  
Telephone: 800-338-9579 • Fax: 866-597-6105**

TMB Substrate Solution. If the solution is blue before use, DO NOT USE IT!

1. Add 100  $\mu$ l of TMB Substrate Solution into each well.
2. Allow the enzymatic color reaction to develop at room temperature (20-25°C) in the dark for 30 minutes. Do NOT cover plate with a plate sealer. The substrate reaction yields a blue solution.
3. After 30 minutes, stop the reaction by adding 100  $\mu$ l of Stop Solution to each well. Tap plate gently to mix. The solution in the wells should change from blue to yellow.

#### **Absorbance Measurement**

**Note:** Evaluate the plate within 30 minutes of stopping the reaction.

1. Wipe underside of wells with a lint-free tissue.
2. Measure the absorbance on an ELISA plate reader set at 450 nm.

#### **Calculation of Results**

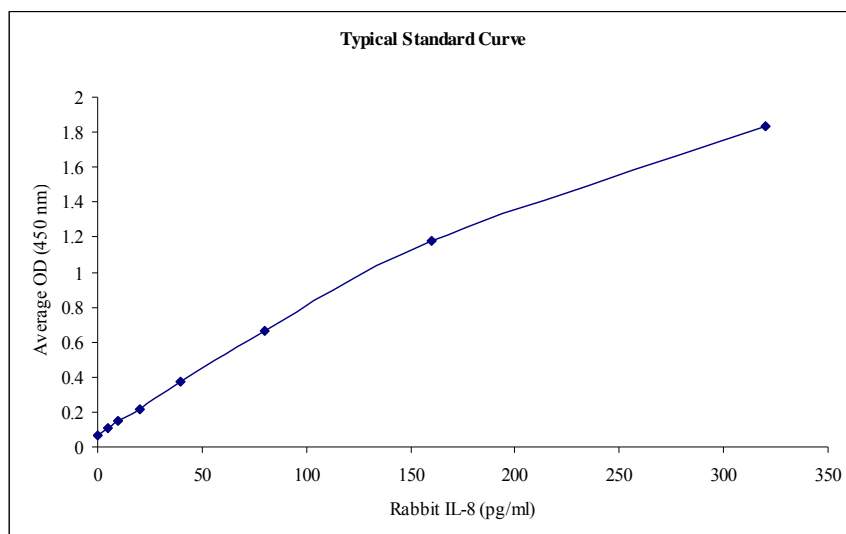
- Duplicate absorbance values should be within 10% of each other. Care should be taken when interpreting data with differences in absorbance values greater than 10%.
1. Prepare a standard curve to determine the amount of Rabbit IL-8 in an unknown sample. Plot the average absorbance obtained for each standard concentration on the vertical (Y) axis versus the corresponding Rabbit IL-8 concentration on the horizontal (X) axis using graph paper or curve-fitting software.
  2. Calculate the Rabbit IL-8 concentration in unknown samples using the prepared standard curve. Determine the amount of Rabbit IL-8 in each unknown sample by noting the Rabbit IL-8 concentration (X axis) that correlates with the absorbance value (Y axis) obtained for the unknown sample.
  3. If the sample was diluted, multiply the Rabbit IL-8 concentration obtained by the dilution factor to determine the amount of Rabbit IL-8 in the undiluted sample.

**Bethyl Laboratories, Inc. • [www.bethyl.com](http://www.bethyl.com)  
Telephone: 800-338-9579 • Fax: 866-597-6105**

## **Performance Characteristics**

### **Typical Standard Curve**

- This typical standard curve was generated using the Rabbit IL-8 ELISA Kit Protocol. This standard curve is for demonstration only. A standard curve must be generated for each assay.



**Assay Range:** 320-5 pg/ml

- Suggested standard curve points are 320 pg/ml, 160 pg/ml, 80 pg/ml, 40 pg/ml, 20 pg/ml, 10 pg/ml, 5 pg/ml, and 0 pg/ml.

### **Representative Data**

- PBMCs were harvested by ficoll density gradient from day old whole blood collected from an apparently healthy rabbit. The PBMCs were suspended at  $1 \times 10^6$  cells/ml in RPMI medium containing 10% fetal bovine serum. PBMCs were stimulated with phytohemagglutinin (PHA; 10  $\mu$ g/ml), staphylococcal enterotoxin B (SEB; 5  $\mu$ g/ml) or phorbol 12-myristate 13-acetate (PMA; 10 ng/ml) and ionomycin (500 ng/ml). Cell-free supernatants were harvested following three days stimulation and run in the Rabbit IL-8 ELISA. The levels of Rabbit IL-8 detected are as follows:

**Bethyl Laboratories, Inc. • [www.bethyl.com](http://www.bethyl.com)**

**Telephone: 800-338-9579 • Fax: 866-597-6105**

<b>Stimulant</b>	<b>Rabbit IL-8 (pg/ml)</b>
Unstimulated	176
PHA	13,333
SEB	33,363
PMA/Ionomycin	5,758

### **Warranty**

Products are warranted by Bethyl Laboratories, Inc. to meet stated product specifications and to conform to label descriptions when used, handled and stored according to instructions. Unless otherwise stated, this warranty is limited to six months from date of sale. Bethyl Laboratories sole liability for the product is limited to replacement of the product or refund of the purchase price. Bethyl Laboratories products are supplied for research applications. They are not intended for medicinal, diagnostic or therapeutic use. The products may not be resold, modified for resale or used to manufacture commercial products without prior written approval from Bethyl Laboratories, Inc.

Rev 110622

**Bethyl Laboratories, Inc. • [www.bethyl.com](http://www.bethyl.com)  
Telephone: 800-338-9579 • Fax: 866-597-6105**

**Plate Templates**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Bethyl Laboratories, Inc. • [www.bethyl.com](http://www.bethyl.com)  
Telephone: 800-338-9579 • Fax: 866-597-6105