

### Description

The IgE Responsive Luciferase Reporter RBL-2H3 Cell Line is an engineered rat-derived basophilic leukemia RBL-2H3 cell expressing a firefly luciferase reporter under the control of NFAT (factor nuclear factor of activator T cells) response element and expressing human Fc epsilon receptor I alpha (human FcεRIα; hFCER1A NM\_002001.4). With this cell line, IgE (immunoglobulin E) mediated signaling can be monitored by measuring luciferase activity.

This cell line has been validated for its response to recombinant human IgE and inhibition by the anti-IgE antibody Omalizumab.

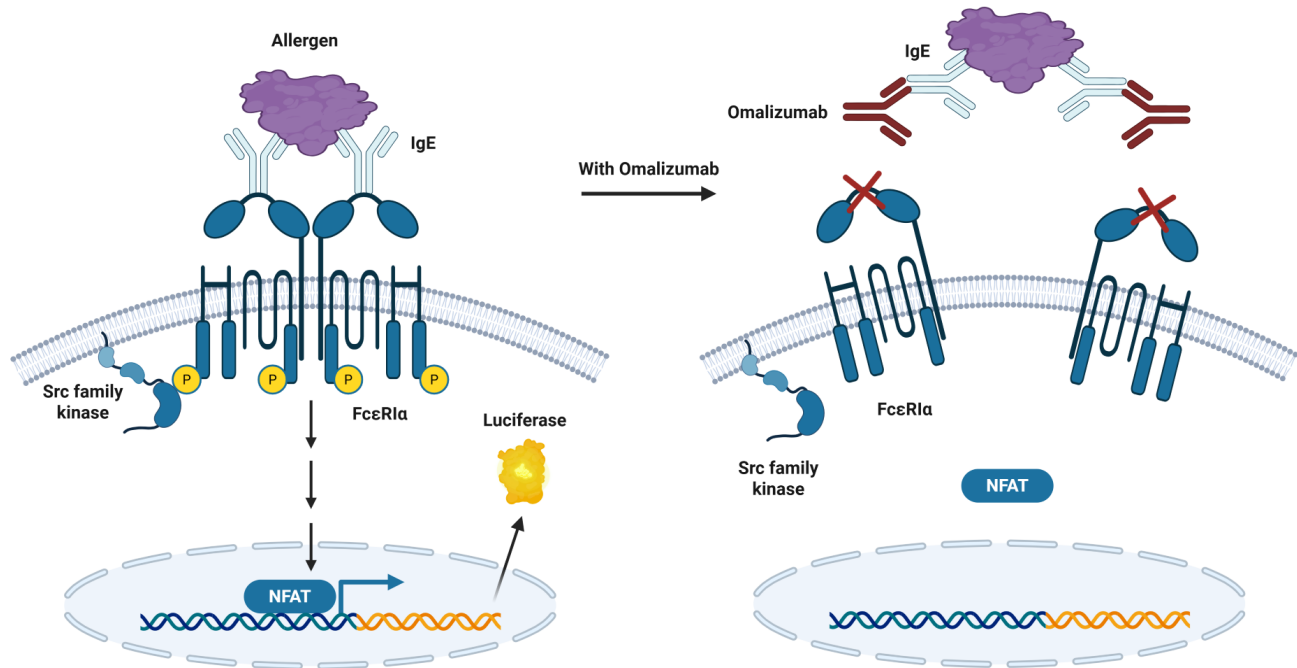


Figure 1: Illustration of the mechanism underlying pathway activation by IgE/allergen-mediated clustering of FcεRIα (left). Omalizumab binds to the constant region of IgE, blocking IgE binding to cellular receptor FcεRIα, thereby preventing clustering and activation of downstream NFAT signaling (right).

### Background

Anti-IgE therapy has emerged as an effective treatment for allergic diseases by targeting immunoglobulin E (IgE), a key mediator of allergic reactions. By binding to circulating IgE, anti-IgE monoclonal antibodies (such as Omalizumab) prevent IgE from interacting with its high-affinity receptor FcεRI on mast cells and basophils. This reduces cell activation and the release of inflammatory mediators that trigger allergic symptoms. Clinical studies have demonstrated that anti-IgE therapy significantly decreases the frequency and severity of allergic responses in conditions such as allergic asthma, chronic spontaneous urticaria, and other IgE-mediated disorders. Overall, anti-IgE therapy provides an effective targeted approach for controlling allergic inflammation and improving patient outcomes.

### Application(s)

- Monitor human IgE mediated signaling in RBL-2H3 cells.
- Screen for anti-IgE antibody.

**Materials Provided**

Components	Format
2 vials of frozen cells	Each vial contains $\geq 1 \times 10^6$ cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

**Parental Cell Line**

RBL-2H3, Rat, Basophilic Leukemia, adherent.

**Mycoplasma Testing**

The cell line has been screened to confirm the absence of Mycoplasma species.

**Materials Required but Not Supplied**

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

*Media Required for Cell Culture*

Name	Ordering Information
Thaw Medium 11	BPS Bioscience #79976
Growth Medium 11B	BPS Bioscience #84112

**Storage Conditions**

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a  $-80^{\circ}\text{C}$  freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

**Media Formulations**

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.



Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene(s) of interest.

Cells should be grown at  $37^{\circ}\text{C}$  with 5%  $\text{CO}_2$ . BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

*Media Required for Cell Culture*

*Thaw Medium 11 (BPS Bioscience #79976):*

DMEM medium supplemented with 10% heat-inactivated FBS, 1% Penicillin/Streptomycin, 1% GlutaMax.

*Growth Medium 11B (BPS Bioscience #84112):*

DMEM medium supplemented with 10% heat-inactivated FBS, 1% Penicillin/Streptomycin plus 500  $\mu\text{g}/\text{ml}$  of Geneticin and 0.5  $\mu\text{g}/\text{ml}$  Puromycin.

*Materials Required for Cellular Assay*

Name	Ordering Information
Assay Medium: Thaw Medium 11	BPS Bioscience #79976
Growth Medium 11B	BPS Bioscience #84112
Recombinant human IgE Lambda	BioRad #HCA171
Omalizumab	BPS Bioscience #83596
Anti-human IgE antibody, polyclonal	BioRad #STAR147
96-well tissue culture treated white clear-bottom assay plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

**Cell Culture Protocol***Cell Thawing*

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 11.

**Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.**

2. Immediately spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 11.
3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO<sub>2</sub> incubator.
4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 11 and continue growing in a 5% CO<sub>2</sub> incubator at 37°C until the cells are ready to be split.
5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 11B.

*Note: Once RBL-2H3 reach confluency, some cells are likely to detach and float. This is normal. If needed, remove the floating cells by aspirating the medium and wash the culture with phosphate buffered saline (PBS) without Ca<sup>2+</sup>/Mg<sup>2+</sup>.*

*Cell Passage*

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca<sup>2+</sup>/Mg<sup>2+</sup>, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 11B and transfer to a tube.
3. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 11B.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:10 every three days.

#### Cell Freezing

1. Aspirate the medium, wash the cells with PBS without  $\text{Ca}^{2+}/\text{Mg}^{2+}$ , and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 11B and count the cells.
3. Spin down the cells at  $300 \times g$  for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at  $\sim 1 \times 10^6$  cells/ml.
4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.
5. Transfer the vials to liquid nitrogen the next day for long term storage.



Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

#### Validation Data

- The following assays are designed for a 96-well format. To perform the assay in different tissue culture formats, cell number and reagent volumes should be scaled appropriately.
- The experiment should be performed using triplicates.
- Assay A should include “Stimulated”, “Unstimulated Control” and “Background Luminescence” conditions.
- Assay B should include “Stimulated, Antibody”, “Stimulated, No Antibody”, “Unstimulated” and “Background Luminescence” conditions.

#### A. Dose-Response of IgE Responsive Luciferase Reporter RBL-2H3 Cell Line to human IgE.

##### Day 1:

1. Seed IgE Luciferase Reporter RBL-2H3 cells at a density of 30,000 to 40,000 cells per well into a white clear-bottom 96-well cell culture plate in 90  $\mu\text{l}$  of Assay Medium. Leave a few empty wells as “Background Luminescence” control.
2. Incubate at 37°C with 5%  $\text{CO}_2$  for 3 hours.
3. Prepare a serial dilution of human IgE at concentrations 10-fold higher than desired final concentrations in Assay Medium (10  $\mu\text{l}$ / well).
4. Add 10  $\mu\text{l}$  of each dilution to the “Stimulated” wells.
5. Add 10  $\mu\text{l}$  of Assay Medium to the “Unstimulated Control” wells (for Negative Control).
6. Add 100  $\mu\text{l}$  of Assay Medium to “Background Luminescence”.

7. Incubate the cells at 37°C in a 5% CO<sub>2</sub> incubator overnight.

### Day 2:

1. Prepare a solution of polyclonal anti-human IgE antibody at 5 µg/ml in Assay Medium (100 µl/well).
2. Remove the medium from the “Stimulated” and “Unstimulated” wells.
3. Wash the cells once using 150 µl of PBS without Ca<sup>2+</sup>/Mg<sup>2+</sup>.
4. Add 100 µl of diluted polyclonal anti-human IgE antibody to each well.
5. Incubate the cells at 37°C in a 5% CO<sub>2</sub> incubator for 3 ~ 4 hours.
6. Add 100 µl of ONE-Step™ Luciferase reagent to each well.
7. Shake the plate gently at room temperature (RT) for 10 to 15 minutes.
8. Measure the luminescence using a luminometer.

### Data Analysis

Subtract the average background luminescence (cell-free wells) from the luminescence reading of all wells. The fold induction of luciferase reporter expression is the average background-subtracted luminescence of the stimulated wells divided by the average background-subtracted luminescence of the unstimulated control wells.

$$\text{Fold induction} = \frac{(\text{luminescence of stimulated cells} - \text{average background})}{(\text{average luminescence of unstimulated cells} - \text{average background})}$$

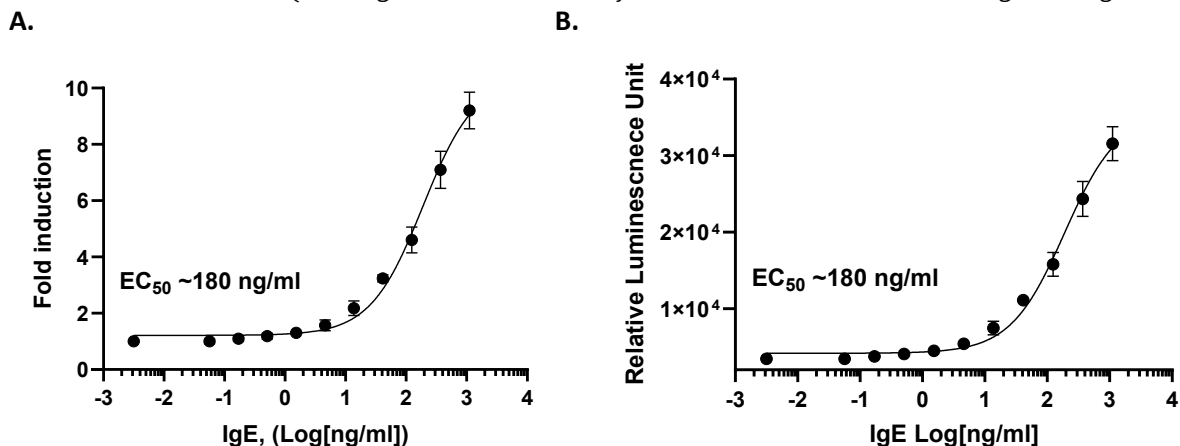


Figure 2. Response to recombinant human IgE in IgE Responsive Luciferase Reporter RBL-2H3 Cell Line.

IgE Responsive Luciferase Reporter RBL-2H3 cells were sensitized with increasing concentrations of IgE overnight and stimulated with a polyclonal anti-human IgE antibody for ~ 4 hours. Luciferase activity was measured using ONE-Step™ Luciferase Assay System (#60690). Unstimulated cells were used as control. Data is shown as fold induction (A) and Relative Luminescence Units (B).

**B: Dose-Response of IgE Responsive Luciferase Reporter RBL-2H3 Cell Line to an anti-IgE neutralizing antibody (e.g. Omalizumab).**

**Day 1:**

1. Seed IgE Luciferase Reporter RBL-2H3 cells at a density of 30,000 to 40,000 cells per well into white clear-bottom 96-well cell culture plate in 80  $\mu$ l of Assay Medium. Leave a few empty wells as “Background Luminescence” control.
2. Incubate at 37°C with 5% CO<sub>2</sub> for 3 hours.
3. Prepare a serial dilution of anti-human IgE neutralizing antibody (e.g. Omalizumab) at concentrations 10-fold higher than desired final concentrations in Assay Medium (10  $\mu$ l/well).
4. Dilute recombinant human IgE at a concentration 10-fold higher than the desired testing concentration in Assay Medium (e.g. prepare a 1  $\mu$ g/ml solution to test IgE at 100 ng/ml) (10  $\mu$ l/well).
  - a. Combine 10  $\mu$ l of diluted IgE and 10  $\mu$ l of the serially diluted anti-IgE neutralizing antibody for each “Stimulated, Antibody” well.
  - b. Combine 10  $\mu$ l of diluted IgE and 10  $\mu$ l of Assay Medium for each “Stimulated, No Antibody” well.
  - c. Incubate the mixture for 1 hour at RT.
5. Add 20  $\mu$ l of the respective mixture to the “Stimulated, Antibody” and “Stimulated, No Antibody” wells.
6. Add 20  $\mu$ l of Assay Medium to the “Unstimulated” wells.
7. Incubate at 37°C with 5% CO<sub>2</sub> incubator overnight.

**Day 2:**

1. Prepare a solution of polyclonal anti-human IgE antibody at 5  $\mu$ g/ml in Assay Medium (100  $\mu$ l/well).
2. Remove the medium from the wells.
3. Wash the cells once using 150  $\mu$ l of PBS without Ca<sup>2+</sup>/Mg<sup>2+</sup>.
4. Add 100  $\mu$ l of diluted polyclonal anti-human IgE antibody to each well.
5. Incubate the cells at 37°C in a 5% CO<sub>2</sub> incubator for 3 ~ 4 hours.
6. Add 100  $\mu$ l of ONE-Step™ Luciferase reagent to each well.
7. Shake the plate gently at RT for 10 ~ 15 minutes.
8. Measure the luminescence using a luminometer

**Data Analysis**

Subtract the average luminescence of the “Background Luminescence” wells from the luminescence reading of all wells. The percent luminescence is the average background-subtracted luminescence of the neutralizing antibody-treated wells divided by the average background-subtracted luminescence of the “Positive Control” wells (IgE only, no neutralizing antibody) multiplied by 100.

$$\% \text{ Luminescence} = \left( \frac{(\text{luminescence of Test Antibody cells} - \text{average Background})}{(\text{average luminescence of Positive Control} - \text{average Background})} \right) \times 100$$

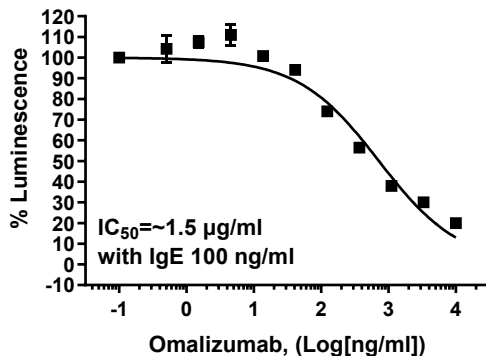


Figure 3. Effect of the neutralizing antibody Omalizumab on the response to recombinant human IgE in IgE Responsive Luciferase Reporter RBL-2H3 Cell Line.

Human IgE was incubated with increasing concentrations of Omalizumab for 1 hour and added to the IgE Responsive Luciferase Reporter RBL-2H3 cells as described in the protocol before activation with a polyclonal anti-human IgE antibody. Luciferase activity was measured using ONE-Step™ Luciferase Assay System (#60690). Stimulated cells in the absence of antibody were used as control.

Data shown is representative.

**Sequence**

Human FcεR1α (NM\_002001.4)

MAPAMESPTLLCVALLFFAPDGVLA V P Q K P K V S L N P P W N R I F K G E N V T L T C N G N N F F E V S S T K W F H N G S L S E E T N S S L N I V N A K F  
 E D S G E Y K C Q H Q Q V N E S E P V Y L E V F S D W L L L Q A S A E V V M E G Q P L F R C H G W R N W D V Y K V I Y Y K D G E A L K Y W Y E N H N I S I T N A T V  
 E D S G T Y Y C T G K V W Q L D Y E S E P L N I T V I K A P R E K Y W L Q F F I P L L V V I L F A V D T G L F I S T Q Q Q V T F L L K I R T R K G F R L L N P H P K P N P K N  
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**References**

Takagi K., *et al*, 2003 *Biol. Pharm. Bull.* 26(2): 252-255.  
 Wang Y., *et al*, 2022 *Euro. J. Pharm. Sci.* 178: 106292

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*Version 040626*