Description

The Human IL-15 Responsive Luciferase Reporter Ba/F3 Cell Line is a murine Ba/F3 cell line engineered to express both human CD122 (hIL2RB, NM_000878.5) and CD132 (hIL2RG, NM_000206.3) separated by a self-cleaving P2A peptide. The construct was delivered by lentiviral transduction of STAT5 Luciferase Reporter Ba/F3 cells (#79772), which express a firefly luciferase reporter driven by STAT5 (Signal transducer and activator of transcription 5) response elements located upstream of the minimal TATA promoter. After activation by IL-15, the endogenous transcription factor STAT5 binds to the response elements, inducing transcription of the luciferase reporter.

This cell line has been validated to respond to human IL-15 and IL-2. Functional validation demonstrates that IL-15-induced luciferase activity can be inhibited by anti-IL15 neutralizing antibody Ordesekimab.

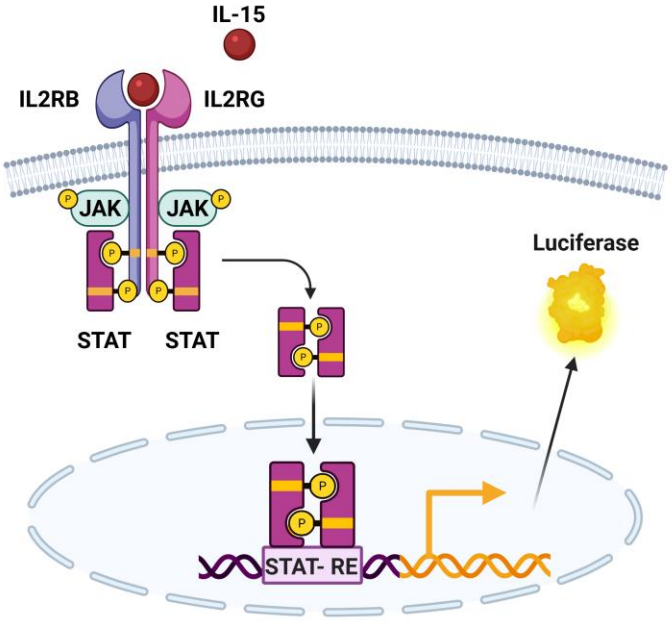

Figure 1: Illustration of the mechanism of Human IL-15 Luciferase Reporter Ba/F3 Cell Line.

IL-2 (interleukin 2) and IL-15 (interleukin 15) both bind to and signal through a complex composed of IL-2/IL-15 receptor beta chain (CD122) and the common gamma-chain (gamma-C, CD132). Activation of the receptor activates downstream JAK (janus kinase) tyrosine kinases, which phosphorylate the transcription factor STAT5. STAT5 phosphorylation triggers the formation of a homodimer and translocation to the nucleus, where it can activate the transcription of the Firefly luciferase reporter driven by STAT5 response elements present in the promoter.

Background

IL-15 is a proinflammatory cytokine mainly produced in dendric cells (DC) and monocytes. It remains associated with IL-15Rα (IL-15 receptor alpha subunit) on the surface of DC and monocytes and binds to the CD122 (IL-12/IL-15 receptor β)/CD132 (γ subunit) heterodimeric receptor present at the surface of various target immune cells, including $CD8^+$ memory T cells, resulting in their proliferation and differentiation. IL-15 is a cytokine of interest in cancer therapy and in immunology. It is produced in cells as a stable heterodimer composed of the IL-15 polypeptide single chain and IL-15Rα, which functions not as receptor but a binding partner that allows transport to the cell membrane. Although there is no indication that unassociated IL-15 exists *in vivo*, soluble recombinant IL-15 and its analogs have been examined for their therapeutic potential.

Application
Screen and characterize modulators of IL-15-triggered signaling.

Materials Provided

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

Parental Cell Line
Ba/F3, mouse IL-3-dependent pro-B cell line, suspension.

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 8	BPS Bioscience #79652
Mouse Interleukin-3 Recombinant	BPS Bioscience #90189
Growth Medium 8E	BPS Bioscience #83556

Materials Required for Cellular Assay

Name	Ordering Information
Assay Medium: Thaw Medium 8	BPS Bioscience #79652
Human IL-15 Recombinant Protein	ThermoFisher #200-15
Human IL-2 Recombinant Protein	ThermoFisher #200-02
Ordesekimab	MedChemExpress #HY-P99410
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
White, clear-bottom 96-well tissue culture plate	Corning #3610
Luminometer	

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°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 these 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 of interest. Cells should be grown at 37℃ with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Note: Mouse IL-3 is essential for Ba/F3 cell maintenance. Thaw Medium 8 and Growth Medium 8E do not contain IL-3.

Complete Thaw Medium: Thaw Medium 8 (BPS Bioscience #79652) with mouse IL-3 (BPS Bioscience #90189):
RPMI 1640 medium (ATCC modification) supplemented with 10% heat-inactivated FBS, 1% Penicillin/Streptomycin and 5 ng/ml of mouse IL-3.

Complete Growth Medium: Growth Medium 8E (BPS Bioscience #83556) with mouse IL-3 (BPS Bioscience #90189):
RPMI 1640 medium (ATCC modification) supplemented with 10% heat-inactivated FBS, 1% Penicillin/Streptomycin, 1 µg/ml of Puromycin, 1,000 µg/ml G418 and 5 ng/ml of mouse IL-3.

Media Required for Functional Cellular Assay

Thaw Medium 8 (BPS Bioscience #79652):
RPMI 1640 medium (ATCC modification) supplemented with 10% heat-inactivated FBS, 1% Penicillin/Streptomycin.

Cell Culture Protocol

Cell Thawing

1. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.

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

2. Using a 10 ml serological pipette, slowly add 10 ml of Thaw Medium 8 to the conical tube containing the cells. The Thaw Medium should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

3. Immediately spin down the cells at 300 *x g* for 5 minutes.

4. Carefully remove the medium and resuspend the cells in 5 ml of pre-warmed Complete Thaw Medium.

5. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.

6. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Complete Thaw Medium and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

7. Cells should be passaged before they reach a density of 2 x 10^6 cells/ml. At first passage and subsequent passages, use Complete Growth Medium.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, but no less than 0.05 x 10^6 cells/ml, in Complete Growth Medium. The sub-cultivation ratio should maintain the cells between 0.05 x 10^6 cells/ml and 2 x 10^6 cells/ml.

Cell Freezing

1. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Cell Freezing Medium (BPS Bioscience #79796) at a density of ~2 x 10^6 cells/ml.

2. 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.

3. Transfer the vials to liquid nitrogen the next day for storage.

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

Validation Data

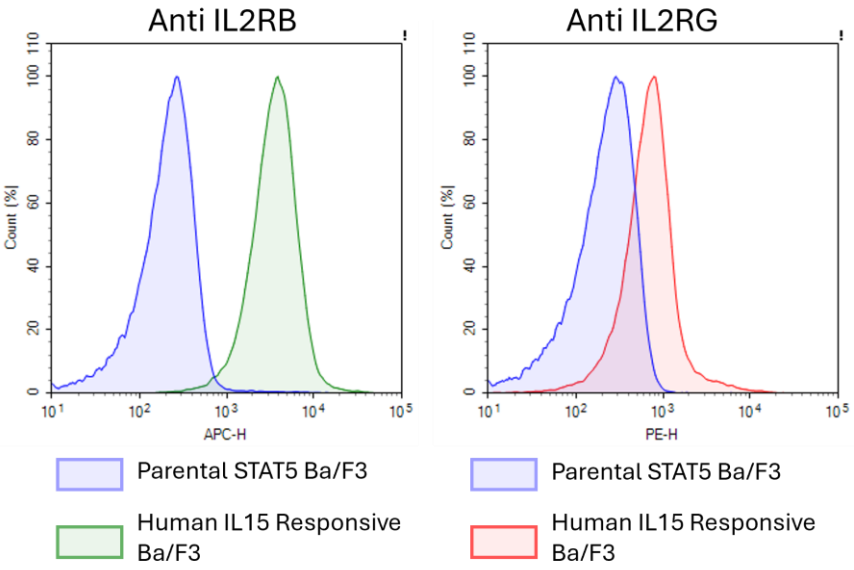

Figure 2: Flow cytometry analysis of human IL2RB and IL2RG expression in Human IL-15 Responsive Luciferase Reporter Ba/F3 Cell Line.
Human IL-15 Responsive Luciferase Reporter Ba/F3 cells and parental STAT5 Luciferase Reporter Ba/F3 cells (#79772) were stained with either APC anti-human CD122 (IL2Rβ) Antibody (BioLegend #339008) or PE anti-human CD132 (common γ chain) Antibody (BioLegend #314603) and analyzed by flow cytometry. The y axis represents the % of cells, while the x axis represents the fluorophore intensity.

Functional Validation

- The following assays are designed either for a 96-well or a 364-well format. To perform the assay in different tissue culture formats, cell number and reagent volume should be scaled appropriately.
- The assay should be performed in triplicate.
- The assay media should not contain mouse IL-3 (**Assay Medium: Thaw Medium 8 (no mouse IL-3)**).

A. Agonist evaluation in Human IL-15 Responsive Luciferase Reporter Ba/F3 Cell Line - 96-well format (*384-well format*)

- This experiment measures the effect of an agonist on reporter activation.
- The assay should include "Stimulated", "Unstimulated" (negative control, no agonist) and "Background Luminescence" (no cells) conditions.

1. Culture IL-15 Responsive Luciferase Reporter Ba/F3 cells in Growth Medium 8E until they reach a density of ~ 2 x 10^6 cells/ml.

2. Collect the cells from culture by centrifugation at 300 *x g* for 5 minutes and wash the cells with Assay Medium.

3. Collect the cells and wash again with Assay Medium.

4. Centrifuge and resuspend the cell pellet in Assay Medium, and count the cell density.

5. Seed cells at a density of 30,000 ~ 40,000 cells/well (*4,000 ~ 5,000 cells/well for a 384-well plate*) in 90 µl (*45 µl for a 384-well*) of Assay Medium into a white, clear-bottom 96-well cell culture plate (*white clear-bottom or white opaque bottom 384-well cell culture plate*). Keep a few empty wells as "Background Luminescence" control.

6. Incubate at 37°C with 5% CO_2 for ~20 hours (overnight).

   Note: It is critical to wash the cells twice with Assay Medium (which does not contain mouse IL-3) and starve the cells for ~ 20 hours in Assay Medium to reduce background signal.

7. Prepare a 3-fold increment serial dilution of human IL-15 in Assay Medium at concentrations 10-fold higher than the desired final concentrations (10 µl/well for a 96-well plate or *5 µl/well for a 384-well plate*).

8. Add 10 µl (*5 µl for a 384-well*) of each dilution to the "Stimulated" wells.

9. Add 10 µl (*5 µl for a 384-well*) of Assay Medium to the "Unstimulated" wells.

10. Add 100 µl (*50 µl for a 384-well*) of Assay Medium to the "Background Luminescence" wells.

11. Incubate at 37°C with 5% CO_2 for ~ 5 hours.

12. Add 100 µl/well (*50 µl for a 384-well*) of ONE-Step™ Luciferase reagent.

13. Incubate at Room Temperature (RT) for ~15 minutes.

14. Measure luminescence using a luminometer.

15. Data Analysis: Subtract the background luminescence from the luminescence reading of all the wells. The fold induction of STAT5 luciferase reporter expression is the background-subtracted luminescence of stimulated cells divided by the background-subtracted luminescence of unstimulated control wells.

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

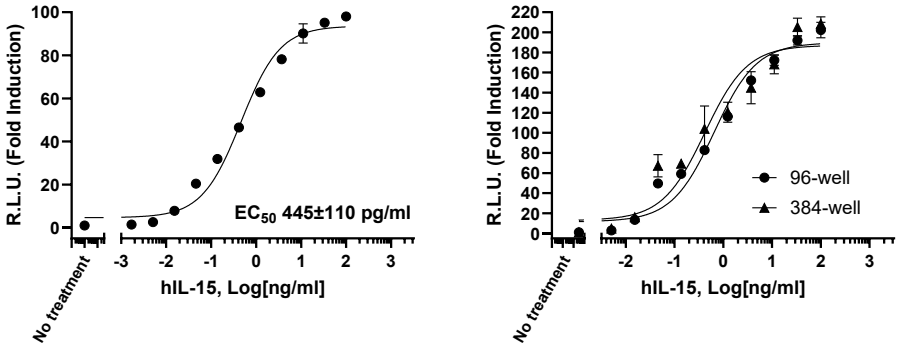

Figure 3: Dose-dependent response of Human IL-15 Responsive Luciferase Reporter Ba/F3 Cell Line to human IL-15.
Human IL-15 Responsive Luciferase Reporter Ba/F3 cells were incubated with increasing concentrations of recombinant human IL-15 for 5-6 hours and luciferase activity was measured using the One-Step™ Luciferase Assay System (#60690). The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing luciferase activity of hIL-15 stimulated cells against the activity of cells without agonist (unstimulated).

B. Inhibition of IL-15 stimulation by an anti-IL15 neutralizing antibody in Human IL-15 Responsive Luciferase Reporter Ba/F3 Cell Line

- This experiment measures the effect of compounds, such as neutralizing antibodies, on the stimulation by IL-15.
- The assay should include “Stimulated, No Compound”, “Unstimulated, No Compound”, “Background Luminescence” and “Test Compound” conditions.

1. Culture the IL-15 Responsive Luciferase Reporter Ba/F3 cells in Growth Medium 8E until they reach a density of ~ 2 x 10^6 cells/ml.

2. Collect the cells from culture by centrifugation at 300 *x g* for 5 minutes and wash the cells with Assay Medium.

3. Collect the cells and wash again with Assay Medium.

4. Centrifuge and resuspend the cell pellet in Assay Medium and count the cell density.

5. Seed cells at a density of 30,000 ~ 40,000 cells/well in 80 µl of Assay Medium into a white, clear-bottom 96-well cell culture plate. Keep a few empty wells as "Background Luminescence" control.

6. Incubate at 37°C with 5% CO_2 for ~20 hours (overnight).

   Note: It is critical to wash the cells twice with Assay Medium Medium (which does not contain mouse IL-3) and starve the cells for ~ 20 hours in Assay Medium reduce background signal.

7. Next day, prepare a 3-fold increment serial dilution of Test Compound (example, neutralizing antibody) in Assay Medium at concentrations 10-fold higher than the desired final concentrations (e.g. 50 µl per well).

8. Add 50 µl to the wells of a new plate that correspond to the "Test Compound" conditions.

9. Add 50 µl of Assay Medium to the "Stimulated, no Compound" wells of the new plate.

10. Dilute human IL-15 in Assay Medium at 100 ng/ml (50 µl/well).

11. Add 50 µl of diluted IL-15 to the "Test Compound" and "Stimulated, no Compound" well of the new plate.

12. Add 100 µl of Assay Medium to the "Unstimulated, no Compound" wells of the new plate.

13. Incubate the antibody/IL-15 mixture for 1 hour at RT.

14. After 1 hour incubation, add 20 µl from the antibody/IL-15 mixture plate to the corresponding wells containing cells.

15. Add 100 µl of Assay Medium to the Assay Medium to the "Background Luminescence" wells.

16. Incubate at 37°C with 5% CO_2 for ~ 5 hours.

17. Add 100 µl/well of ONE-Step™ Luciferase reagent.

18. Incubate at RT for ~15 minutes.

19. Measure luminescence using a luminometer.

20. Data Analysis: Subtract the background luminescence from the luminescence reading of all the wells. The Percent Luminescence of STAT5 luciferase reporter expression is the background-subtracted luminescence of compound-treated stimulated cells divided by the background-subtracted luminescence of unstimulated control wells, multiplied by 100.

$$Percent\ Luminescence = \left(\frac{luminescence\ of\ compound\ treated\ cells - background}{luminescence\ of\ unstimulated, no\ compound\ treated\ cells - background}\right) x100$$

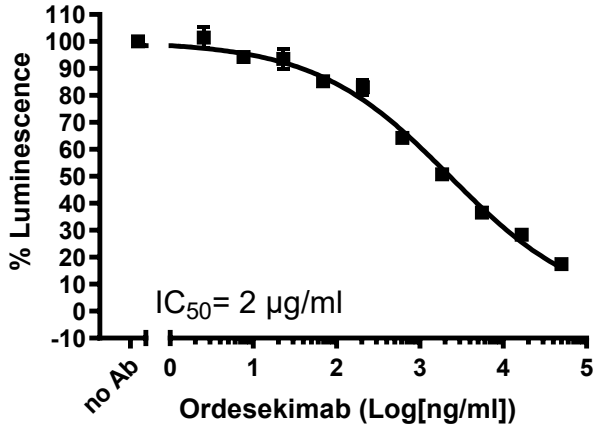

Figure 4: Dose-dependent response of Human IL-15 Responsive Luciferase Reporter Ba/F3 Cell Line to Anti-hIL15 Neutralizing Antibody, Ordesekimab.
Human IL-15 Responsive Luciferase Reporter Ba/F3 cells were incubated with increasing concentrations of Ordesekimab, in the presence of human IL-15, and luciferase activity was measured using the One-Step™ Luciferase Assay System (#60690). Signal from the well stimulated by human IL-15 in the absence of Ordesekimab was set at 100 %, while the wells without IL-15 was defined as 0%.

C. Agonist evaluation in Human IL-15 Responsive Luciferase Reporter Ba/F3 Cell Line – "Thaw-and-Use Format"

- This experiment measures the effect of an agonist on reporter activation immediately after thawing the cells.
- It is recommended that a "mouse IL-3 free" cell bank is made for use in a 'Thaw-and-Use Format", within the recommended passage number for this cell line (see protocol below).

Cell Banking

1. Culture the IL-15 Responsive Luciferase Reporter Ba/F3 cells in Growth Medium 8E until they reach a density of ~ 2 x 10^6 cells/ml.

2. Collect the cells from culture by centrifugation at 300 *x g* for 5 minutes and wash the cells with Assay Medium.

3. Centrifuge, resuspend the cell pellet in Assay Medium, and count the number of cells.

4. Collect the cells by centrifugation again and remove the medium.

5. Resuspend the cell pellet in 4°C Cell Freezing Medium (BPS Bioscience #79796) at a density of 4 ~ 5 x 10^6 cells/ml.

6. 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.

7. Transfer the vials to liquid nitrogen the next day for long term storage.

Thaw-and-Use

1. On the day of the experiment, 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 Assay Medium (no mIL-3.)

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

8. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 10 ml of pre-warmed Assay Medium.

9. Add 90 µl of cell suspension/well to a white, clear-bottom 96-well cell culture plate. Keep a few empty wells as "Background Luminescence" control.

10. Prepare a 3-fold increment serial dilution of human IL-15 in Assay Medium at concentrations 10-fold higher than the desired final concentrations (10 µl/ well).

11. Add 10 µl of each dilution to the "Stimulated" wells.

12. Add 10 µl of Assay Medium to the "Unstimulated" wells.

13. Add 100 µl of Assay Medium to the "Background Luminescence" wells.

14. Incubate at 37°C with 5% CO_2 for ~ 5 hours.

15. Add 100 µl/well of ONE-Step™ Luciferase reagent.

16. Incubate at RT for ~15 minutes.

17. Measure luminescence using a luminometer.

18. Data Analysis: Subtract the background luminescence from the luminescence reading of all the wells. The fold induction of STAT5 luciferase reporter expression is the background-subtracted luminescence of stimulated cells divided by the background-subtracted luminescence of unstimulated control wells.

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

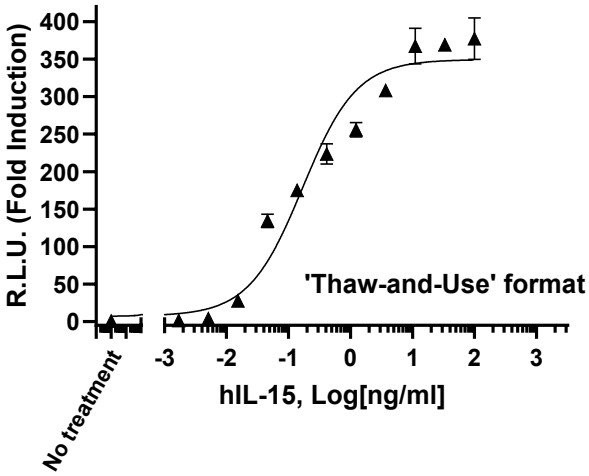

Figure 5: Dose-dependent response of Human IL-15 Responsive Luciferase Reporter Ba/F3 Cell Line to human IL-15 immediately after thawing the cells.
Human IL-15 Responsive Luciferase Reporter Ba/F3 cells were thawed and incubated with increasing concentrations of recombinant human IL-15 for 5-6 hours and luciferase activity was measured using the One-Step™ Luciferase Assay System (#60690). The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing luciferase activity of hIL-15 stimulated cells against the activity of cells without agonist (unstimulated).

Data shown is representative.

References

Bahatt RS., *et. al.*, 2021 *Cancer Immunol Res.* 9(2): 156-169.
Lui G., *et al.*, 2023 *Cells* 12(12):1611.

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Troubleshooting Guide

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