IL-15 Responsive Luciferase Reporter Cell Line

Description

This recombinant Jurkat cell line is a biologically relevant system to measure activation of the IL-15 cytokine receptor by IL-15. The cells were engineered for constitutive expression of human CD122 (IL-15Rβ; IL-2Rβ; NM_000878.4), and conditional expression of firefly luciferase driven by STAT5 response elements located upstream of the minimal TATA promoter. Expression of CD122 allows formation of a functional IL-15 receptor at the surface of Jurkat cells, which naturally express high levels of CD132 (also known as IL-15 receptor subunit γc). Activation of the STAT5 signaling pathway in response to IL-15 or IL-15 analogs can be monitored by measuring luciferase activity.

Background

IL-15 is a proinflammatory cytokine mainly produced in dendritic cells (DC) and monocytes. It remains associated with IL-15Rα on the surface of DC and monocytes, and binds to the CD122/CD132 heterodimeric receptor present at the surface of various target immune cells, including $CD8^+$ memory T cells, resulting in their proliferation and differentiation. 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

1. Characterize recombinant IL-15, IL-15 analogs or other agonists of IL-15R
2. Screen Janus kinase or IL15R inhibitors in a cell-based assay

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)

Host Cell

Jurkat cells (clone E6-1), Human T lymphoblast, 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.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2A	BPS Bioscience #60190

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Materials Required for Cellular Assay

Name	Ordering Information
Recombinant Human IL-15 (rhIL-15)	Peprotech #200-15
RLI (Receptor-linker-IL-15)	BPS Bioscience #101366
Assay Medium: Thaw Medium 2	BPS Bioscience #60184
96-well tissue culture treated, white, clear-bottom assay plate	Corning #3610
ONE-Step™ luciferase assay system	BPS Bioscience #60690
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, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. 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 for maintaining the presence of the transfected gene(s) over passages.
Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 2 (BPS Bioscience #60184):
RPMI1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2A (BPS Bioscience #60190):
RPMI1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 200 µg/ml of Hygromycin and 1 mg/ml Geneticin.

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 2 **(no Geneticin or Hygromycin)**.
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 2 **(no Geneticin or Hygromycin)**.
3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.
4. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Thaw Medium 2 **(no Geneticin or Hygromycin)**, and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they reach a density of 2 x 10^6 cells/ml. At first passage and subsequent passages, use Growth Medium 2A **(contains Geneticin and Hygromycin)**.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, at no less than 0.2 x 10^6 cells/ml of Growth Medium 2A **(contains Geneticin and Hygromycin)**. The sub-cultivation ratio should maintain the cells between 0.2 x 10^6 cells/ml and 2 x 10^6 cells/ml.

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

Validation Data

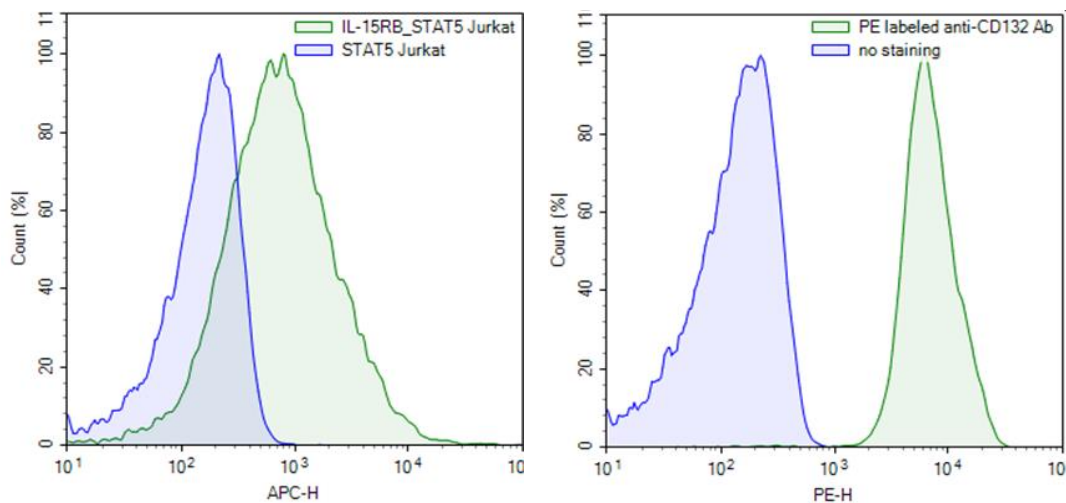

Figure 1. Expression levels of transfected CD122 (IL-15Rβ) and of endogenous CD132. **Left**: IL-15 Responsive Luciferase Reporter Jurkat cells (green) and parental STAT5 Luciferase Jurkat cells (blue) were stained with an APC-conjugated anti-IL-15Rβ antibody (Biolegend, #339008) and analyzed by flow cytometry. **Right**: Intracellular staining of endogeneous CD132 was performed using a PE-labeled anti-CD132 antibody (Biolegend, #314603) and analyzed by flow cytometry. The CD132-specific staining in IL-15 Responsive Luciferase Reporter Cell Line (green) is compared to a no staining control (blue).

Assay Performance

The following assays are designed for a 96-well format. To perform the assay in different tissue culture formats, cell number and reagent volume should be scaled appropriately.

These assays use Thaw Medium 2 (BPS Bioscience #60184) as assay medium.

A. Evaluation of *rh*IL-15 and RLI (receptor-linker-IL-15)

1. Culture the IL-15 Responsive Luciferase Reporter cells until they reach a density of approximately 2 x 10^6 cells/ml on the day of the experiment. Harvest the cells by centrifugation at 300 x g for 5 minutes.

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2. After centrifugation, aspirate the growth medium and resuspend the cells in pre-warmed Thaw medium 2 at a density of 1 x 10^6 cells/ml.
3. Dispense 90 µl/well of the resuspended cells in a white clear bottom 96-well plate. Keep a few wells without cells to determine the background luminescence.
4. Prepare an intermediate dilution of *rh*IL-15 (or IL-15 analog) in Thaw medium 2 at a concentration 10-fold higher than the desired final concentration. Add 10 µL of the intermediate solution of *rh*IL-15 (or IL-15 analog) to each well and add 10 µl of Thaw medium 2 in the unstimulated (negative) control well. The total volume in each well is now 100 µl .
5. Incubate the cells at 37°C in a CO_2 incubator for about 5 hours.
6. After 5 hours, perform the luciferase assay using the ONE-Step™ Luciferase Assay System (BPS Bioscience #60690). Add 100 µl of the ONE-Step™ Luciferase reagent per well and rock at room temperature for 15 minutes.

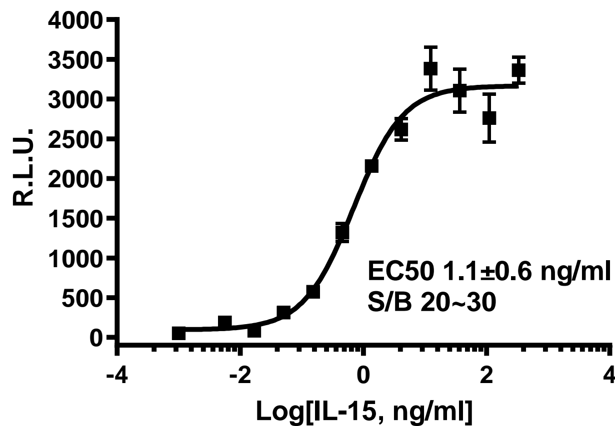

Figure 2. IL-15 agonist stimulation of luciferase activity. The IL-15 cell-based assay was performed with increasing concentrations of *rh*IL-15, and the luciferase reporter activity was measured using the ONE-Step™ Luciferase Assay System reagent (BPS Bioscience #60690). Signal induction was calculated based on the signal obtained from the well containing no *rh*IL-15.

B. Evaluation of Jak inhibitors (*e.g.* Tofacitinib)

1. Culture the IL-15 Responsive Luciferase Reporter cells in Growth Medium 2A until they reach a density of ~2 x 10^6 cells/ml on the day of the experiment.
2. On the day of the experiment, harvest the cells by centrifugation at 300 x g for 5 minutes.
3. After centrifugation, aspirate the growth medium and resuspend the cells in pre-warmed Thaw medium 2 at 1.2 x 10^6 cells/ml density.
4. Dispense 80 µL of the resuspended cells in Thaw medium 2 per well in a white clear bottom 96-well plate.

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5. Prepare an intermediate dilution of Tofacitinib in Thaw medium 2 containing 1% DMSO at a concentration 10-fold higher than the desired final concentration (*e.g. If the desired final concentration of Tofacitinib is 10 nM, prepare 10 µM Tofacitinib in 100% DMSO first. Then dilute 10 µM Tofacitinib 100-fold in Thaw medium 2, resulting in 100 nM Tofacitinib in Thaw medium 2 containing 1% DMSO. This is the intermediate dilution for 10 nM final assay concentration*).
6. Add 10 µl of the diluted Tofacitinib to each well and add 10 µl Thaw medium 2 containing 1% DMSO in the positive and negative control wells.
7. Incubate the cells at 37°C in a CO_2 incubator for 1 hour.
8. Prepare 150 ng/ml *rh*IL-15 in Thaw medium 2 and add 10 µl of the prepared *rh*IL-15 to each well. Add 10 µl of Thaw medium 2 to the negative control.
9. Incubate the cells at 37°C in a CO_2 incubator for ~ 5 hours.
10. After ~ 5 hours, perform the luciferase assay using the ONE-Step Luciferase Assay System (BPS Bioscience #60690). Add 100 µl of the ONE-Step Luciferase reagent per well and rock at room temperature for ~ 15 minutes.

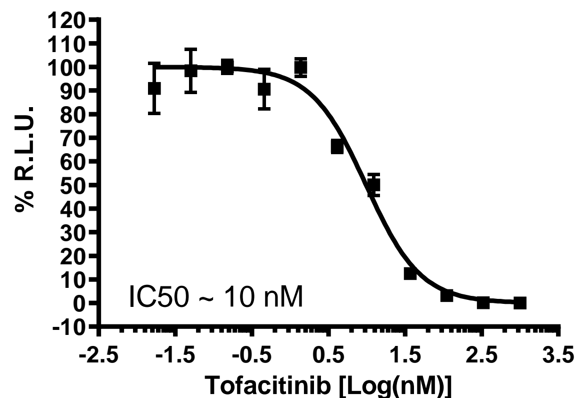

Figure 3. Dose-response of Tofacitinib effect on the IL-15 mediated cellular signaling pathway. Upon binding of IL-15, IL-15Rβ/IL-15Rγ transmits the signal through Janus kinases (JAKs), resulting in phosphorylation of STAT5 and activation of STAT5-mediated gene expression. The percentage of relative luminescence units (R.L.U.) was calculated based on the signal from the positive wells (-Tofacitinib/+*rh*IL-15) and the negative wells (-Tofacitinib/-*rh*IL-15).

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

References

Bahatt RS. *et. al*., *Cancer Immunol Res*. 2021; **9(2):** 156-169.

Related Products

Products	*Catalog #*	*Size*
IL2RB, Avi-FLAG-Tag, Biotin-Labeled HiP™ Recombinant	100314	various
IL2RB, Avi-His-Tag, Biotin-Labeled Recombinant	100428	various
JAK1, GST-Tag Recombinant	40449	10 µg
JAK2 (JH1 domain), His-Tag Recombinant	40450	20 µg
JAK3, His-Tag Recombinant	40452	20 µg
IL-2RB Recombinant (CD122) Recombinant	79655	5 µg

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