Description

NF-κB Luciferase Reporter Raw 264.7 Cell Line is a Raw 264.7 cell line expressing firefly luciferase driven by four copies of the NF-κB response element located upstream of the minimal TATA promoter. After activation by pro-inflammatory cytokines or stimulants of lymphokine receptors, endogenous NF-κB transcription factors bind to the DNA response elements, inducing transcription of the luciferase reporter gene.
This cell line has been functionally validated and responds to TNFα and RANKL.

Background

The role of NF-κB (nuclear factor-κB) activation is well-characterized in canonical (classical) and noncanonical (alternative) signaling pathways of inflammation. Two major forms of innate immune sensors are Toll-like receptors (TLR) and NOD/CATERPILLAR proteins. Mutations in NOD2 (nucleotide-binding oligomerization domain-containing protein 2) have been linked to chronic autoinflammatory and autoimmune diseases, such as Crohn's disease and Blaus syndrome. Studying the canonical and noncanonical NF-κB pathways and the influence of TLR pathways and NOD2 mutations can further our understanding of autoimmune regulation. Raw 264.6 is a murine macrophage cell line useful for studies involving immunoreactivity. The use of a luciferase reporter allows for easy read outs in cellular assays.

Application

- Screen for activators or inhibitors of the NF-κB signaling pathway.
- Screen for activators or inhibitors of the RANKL/RANK pathway.

Materials Provided

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

Parental Cell Line

Raw 264.7, murine macrophage cell line, 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 11A	BPS Bioscience #79977

Materials Used in the Cellular Assay

Name	Ordering Information
Mouse RANKL	R&D Systems #462-TEC-010
Mouse TNFα	R&D Systems #410-MT
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
White clear-bottom 96-well cell culture plate	
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 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°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 11 (BPS Bioscience #79976):

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

Growth Medium 11A (BPS Bioscience #79977):

DMEM medium supplemented with 10% heat-inactivated FBS, 1% GlutaMAX, 1% Penicillin/Streptomycin plus and 700 µg/ml of Genecticin.

Media Required for Functional Cellular Assay

Thaw Medium 11 (BPS Bioscience #79976):

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

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 and incubate at 37°C in a 5% CO2 incubator.

4. After 24 hours of culture, check for cell viability and cell attachment. Change medium to fresh Thaw Medium 11 and continue growing in a 5% CO2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 11A.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 11A and transfer to a tube.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 11A.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:10 every 3-4 days.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 11A and count cells.

3. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 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.

A. Dose response of NF-κB Luciferase Reporter Raw 264.7 Cell Line to RANKL and TNFα.

- The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.
- The experiments should be performed in triplicate.
- The assay should include “Cell-Free Control”, “Unstimulated Control” and “Test Condition” wells.

1. Seed NF-κB Luciferase Reporter Raw 264.7 cells at a density of ~30,000 cells in 90 µl/well of Thaw Medium 11 into a white clear-bottom 96-well cell culture plate. Leave some wells with only Assay Medium for background determination.

2. Incubate cells at 37°C with 5% CO2 overnight.

3. Prepare serial dilutions of mouse RANKL and/or mouse TNFα at 10-fold the final concentration in Thaw Medium 11A (10 µl/well).

4. Add 10 µl of the dilutions to the "Test Condition" cells.

5. Add 100 µl of Thaw Medium 11A to the "Unstimulated Control" and "Cell-Free Control" wells (for determining the background luminescence).

6. Incubate the cells at 37°C in a CO_2 incubator for 5-6 hours.

7. Add 100 µl of ONE-Step™ Luciferase Assay reagent to all wells.

8. Rock at room temperature for ~15 minutes.

9. Measure luminescence using a luminometer.

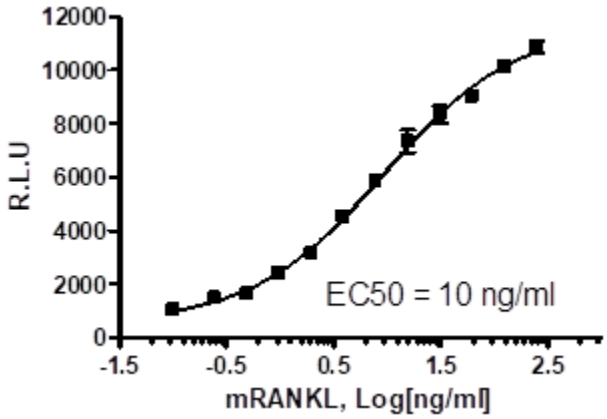

Figure 1. Dose response curve of NF-κB Luciferase Reporter Raw 264.7 Cell Line to mouse RANKL. NF-κB Luciferase Reporter Raw 264.7 cells were treated with increasing concentrations of mouse RANKL. The results are shown as relative luminescence units of luciferase reporter expression.

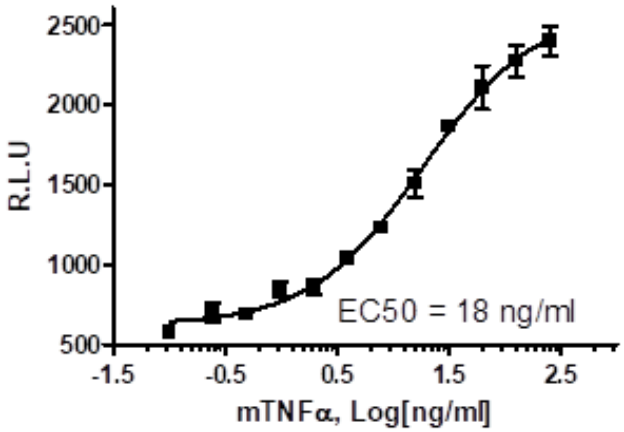

Figure 2. Dose response curve of NF-κB Luciferase Reporter Raw 264.7 Cell Line to mouse TNFα. NF-κB Luciferase Reporter Raw 264.7 cells were treated with increasing concentrations of mouse TNFα. The results are shown as relative luminescence units of luciferase reporter expression.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

Peninnger, J. M., *et al*., 2006 *Trends Mol. Med.* 12(1):17-25.
Baeuerle, P.A., 1998 *Curr Biol*. 8(1):R19-R22.

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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.

Related Products

Products	*Catalog #*	*Size*
NF- κB Reporter (Luc) – THP-1 Cell Line	79645	2 vials
NF- κB Reporter (Luc) – Jurkat Cell Line	60651	2 vials
NF- κB Reporter (Luc) – NIH/3T3 Cell Line	79469	2 vials
NF-κB Reporter Kit	60614	500 reactions
RANKL, His-Tag (Human) Recombinant	71051	25 µg/100 µg
Human Tumor Necrosis Factor-alpha Recombinant	90244	10 µg/50 µg