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Data Sheet

NF- κB Reporter (Luc) – Raw 264.7 Cell line

Catalog #:79978

Product Description

The NF-κB reporter (Luc)-Raw 264.7 cell line is designed for monitoring nuclear factor Kappa B (NF-κB) signal transduction pathways. It contains a firefly luciferase gene 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.

Applications

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

Format

Each vial contains 2 X 10^6 cells in 1 ml of 10% DMSO.

Storage

Store in liquid nitrogen immediately upon receipt.

General Culture Conditions

Thaw Medium 11 (BPS Bioscience #79976): DMEM medium (Hyclone #SH30024.01) supplemented with 10% heat-inactivated FBS (Gibco #26140-079), 1% GlutaMAX (Gibco #35050-061), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Growth Medium 11A (BPS Bioscience #79977): Thaw Medium 11 (BPS Bioscience #79976) plus 700 µg/ml of Genecticin (Life Technologies #11811031).

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 11A.

NF-κB reporter (Luc)-Raw 264.7 cells should exhibit a typical cell division time of 24 hours.

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 11 **(no Genecticin)**, spin down cells, resuspend cells in pre-warmed Thaw Medium 11 **(no Genecticin)**, transfer resuspended cells to T25 flask and culture in 37°C CO_2 incubator. At first passage switch to Growth Medium 11A **(contains Genecticin)**. Cells should be split before they reach complete confluence.

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To passage the cells, rinse cells with phosphate buffered saline (PBS) and enzyme-free cell dissociation buffer (ThermoFisher #13151014), detach cells from culture vessel with enzyme-free cell dissociation buffer, add Growth Medium 11A and transfer to a tube, spin down cells, re suspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ratio: 1:10 every 3-4 days.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS) and enzyme-free cell dissociation buffer (ThermoFisher #13151014), detach cells from culture vessel with enzyme-free cell dissociation buffer. Add Growth Medium 11A and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS). Place at -80°C overnight and place in liquid nitrogen the next day. Alternatively, vials may be placed directly in liquid nitrogen.

Mycoplasma testing
The cell line has been screened using the PCR-based VenorGeM™ Mycoplasma Detection kit (Sigma-Aldrich, #MP0025) to confirm the absence of *Mycoplasma* species.

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

Materials Required but Not Supplied

- mouse RANKL (R&D Systems #462-TEC-010)
- mouse TNFα (R&D Systems #390-TN-010)
- Assay Medium: Thaw Medium 11 (BPS Bioscience #79976)
- Growth Medium 11A (BPS Bioscience #79977)
- 96-well tissue culture treated white clear-bottom assay plate (Corning #3610)
- ONE-Step™ luciferase assay system (BPS Bioscience #60690) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer

A. RANKL (or TNFα) dose response

1. Harvest NF-κB reporter (Luc)-Raw 264.7 cells and seed cells at a density of 30,000 cells per well into white opaque 96-well microplate in 90 µl of assay medium. Incubate cells at 37°C with 5% CO_2 overnight.
2. Add threefold serial dilution of mouse RANKL (or mouse TNFα) in 10 µl of assay medium to RANKL (or TNFα) -stimulated wells.
3. Add 10 µl of assay medium to the unstimulated control wells (for measuring uninduced level of NF-κB reporter activity).
4. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).

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5. Incubate at 37°C with 5% CO_2 for 5-6 hours.
6. Prepare ONE-Step™ Luciferase Assay reagent as directed and add 100 µl per well. Incubate at room temperature for ~5 minutes and measure luminescence using a luminometer. Subtract background luminescence value from all measurements.

Figure 1. Mouse RANKL dose response in NF-κB reporter (Luc)-RAW 264.7 cells. The results are shown as relative luminescence units of luciferase reporter expression.

The EC_{50} of mouse RANKL in this cell line is ~10 ng/ml.

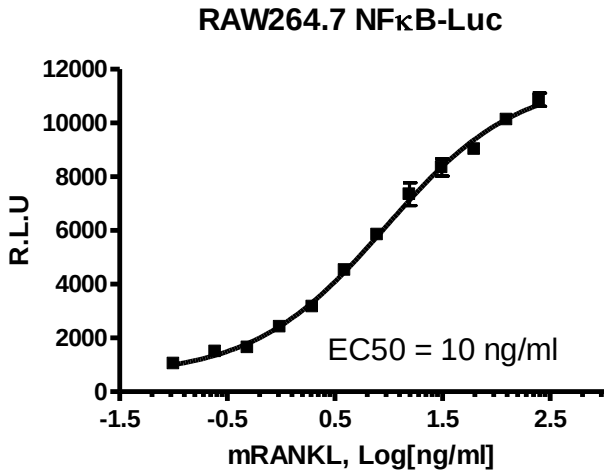

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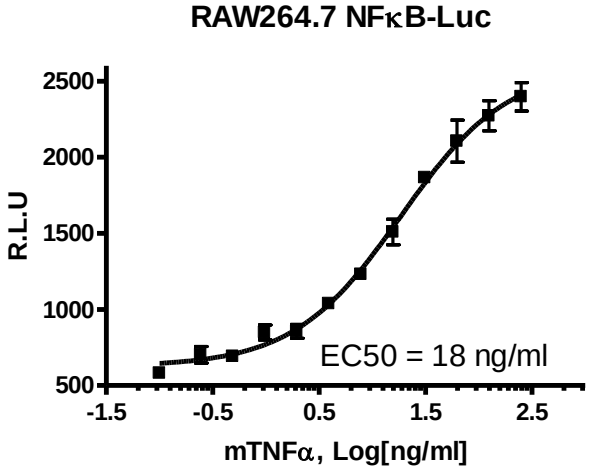
Figure 2. Mouse TNFα dose response in NF-κB reporter (Luc)-RAW 264.7 cells. The results are shown as relative luminescence units of luciferase reporter expression.

The EC_{50} of mouse TNFα in this cell line is ~20 ng/ml.



References

1. Peninnger, J. M., *et al*. (2006) RANKL–RANK signaling in osteoclastogenesis and bone disease. *Trends Mol. Med.* **12(1):**17-25.

2. Baeuerle, P.A. (1998) Pro-inflammatory signaling: last pieces in the NF-κB puzzle? *Curr Biol*. **8(1):**R19-R22.

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Related Products

Product	Cat. #	Size
Human Tumor Necrosis Factor-alpha	90244-A	10 µg
RANKL, His-Tag (Human)	71051	100 µg
Thaw Medium 11	79976	100 ml
Growth Medium 11A	79977	500 ml
NF- κB Reporter (Luc) – THP-1 Cell Line	79645	2 vials
NF- κB Reporter (Luc) – NIH/3T3 Cell Line	79469	2 vials
NF- κB Reporter (Luc) – Jurkat Cell Line	60651	2 vials
NF- κB Reporter (Luc) – A549 Cell Line	60625	2 vials
NF- κB Reporter (Luc) – HCT116 Cell Line	60623	2 vials
NF- B Reporter (Luc) – CHO-K1 Cell Line	60622	2 vials
NF-κB Reporter Kit	60614	500 rxns.
CD27/NF-κB Reporter-Jurkat Cell Line	79509	2 vials
TLR8/NF-κB Reporter-HEK293 Cell Line	60684	2 vials
GITR/NF-κB Reporter-Jurkat Cell Line	60546	2 vials