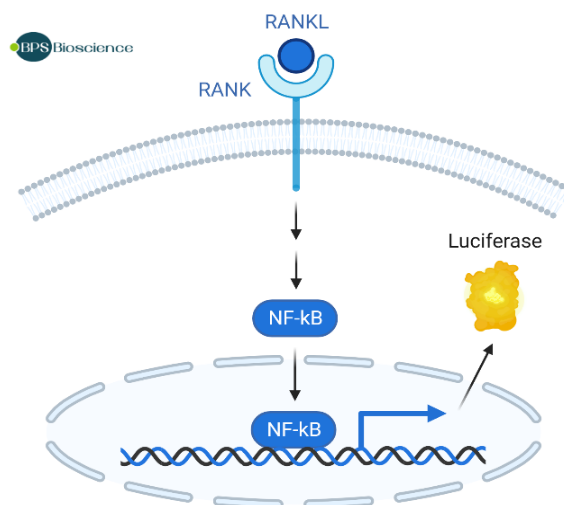
Description

RANK/NF-κB Luciferase Reporter HEK293 Cell Line is a HEK293 cell line expressing the firefly luciferase reporter under the control of Nuclear factor-κB (NF-κB) Response Elements and with constitutive expression of human RANK (Receptor activator of nuclear factor-κB; TNFRSF11A; ref. seq. NM_003839.2). Overexpression of RANK was confirmed by flow cytometry and the cell line was functionally validated for its response to RANK ligand (RANKL), anti-RANKL antibody and IKK-16.



Created with BioRender.com.

Figure 1: Activation of NF-κB-dependent luciferase reporter in the RANK/NF-κB Luciferase Reporter HEK293 Cell Line.

Background

Bone homeostasis relies on the balance between bone formation by osteoblasts and bone resorption by osteoclasts. Appropriate signaling between the two cell types is necessary to maintain bone health. Osteoblasts express RANKL (receptor activator of NF-κB ligand), which binds to the receptor RANK on the surface of osteoclasts. This results in activation of the NF-κB pathway to promote osteoclast survival and proliferation, leading to the resorption of bone. This mechanism is tempered by osteoblast-mediated secretion of OPG (osteoprotegerin) which competes with RANKL for binding to RANK and protects from excessive bone resorption. Therefore, abnormal production of RANKL or OPG can cause osteoporosis and other bone-related disorders. This pathway is also dysregulated by bone-colonizing tumor cells in the first steps of metastasis, making the RANKL/RANK signaling pathway a valuable target for drug discovery and development.

Application

Screen for compound activity on RANK/RANKL/OPG signaling pathway.

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

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent

BPS Bioscience
Our products are for research use only, not for diagnostic or therapeutic use
● bpsbioscience.com ● 858-202-1401 ● info@bpsbioscience.com

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 this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems 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.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1G	BPS Bioscience #79544

Materials Required for Cellular Assay

Name	Ordering Information
Human RANKL	R&D Systems #390-TN-010
Anti-RANKL Neutralizing Antibody	BPS Bioscience #100874
IKK-16 dihydrochloride	Sigma #SML1138
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells will arrive upon 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 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 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1G (BPS Bioscience #79544):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, 400 µg/ml Geneticin, 50 µg/ml Hygromycin B.

Assay Medium: Thaw Medium 1 (BPS Bioscience #60187).

For research use only. Not for human, drug, diagnostic, or therapeutic use.
BPS Bioscience
● bpsbioscience.com ● 858-202-1401 ● info@bpsbioscience.com

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 1.
   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 1.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 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 are fully confluent. At first passage and subsequent passages, use Growth Medium 1G.

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 1G 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 1G.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 once or twice a week.

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 1G and count the 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 ~2×10^6 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 down at least 10 vials of cells at an early passage for future use.

Validation Data

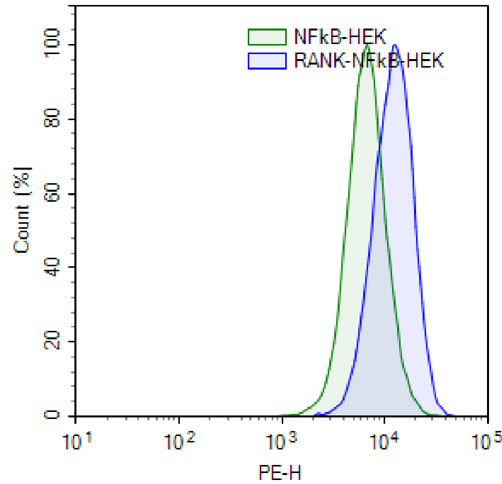


Figure 2. Cell surface expression of RANK in RANK/NF-κB Reporter HEK293 Cell Line.
RANK/NF-κB Luciferase Reporter HEK293 cells (blue) or control NF-κB Luciferase Reporter (Luc) HEK293 cells (BPS Bioscience #60650) (green) were stained with Human RANK/TNFRSF11A PE-conjugated Antibody (R&D Systems #FAB683P) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates the PE intensity.

Functional Validation

- The following assays are designed for 96-well format. To perform the assay in a different format, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- Assay A should include “Stimulated”, “Cell-Free Control” and “Unstimulated Control” conditions.
- Assay B and C should include "Treated”, “Untreated”, “Cell-Free Control” and “Unstimulated Control” conditions.

A. Dose response of RANK/NF-κB Luciferase Reporter HEK293 Cell Line to human RANKL

1. Seed RANK/NF-κB Luciferase Reporter HEK293 cells in 90 µl of Thaw Medium 1 at a density of ~30,000 cells per well into a 96-well white clear-bottom cell culture plate Leave a few wells empty to use as the “Cell-Free Control” (Background Signal).

2. Incubate the cells at 37°C in a CO_2 incubator for ~16 hours.

3. Prepare a two-fold serial dilution of human RANKL in Thaw Medium 1 at concentrations 10-fold higher than the final desired concentrations (10 µl/well).

4. Add 10 µl of serially diluted RANKL to the “Stimulated” wells.

5. Add 10 µl of Thaw Medium 1 to the “Unstimulated Control” wells.

BPS Bioscience
For research use only. Not for human, drug, diagnostic, or therapeutic use.
● bpsbioscience.com ● 858-202-1401 ● info@bpsbioscience.com

6. Add 100 µl of Thaw Medium 1 to the "Cell-Free Control" wells (for determining background luminescence).

7. Incubate the plate at 37°C in a CO_2 incubator for 6 hours.

8. Add 100 µl of the ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 to 30 minutes.

9. Measure luminescence using a luminometer.

10. Data Analysis: Subtract average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of NF-κB 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.

$$Fold\ Induction = \frac{Lumin.of\ stimulated - background}{Lumin.of\ unstimulated - background}$$

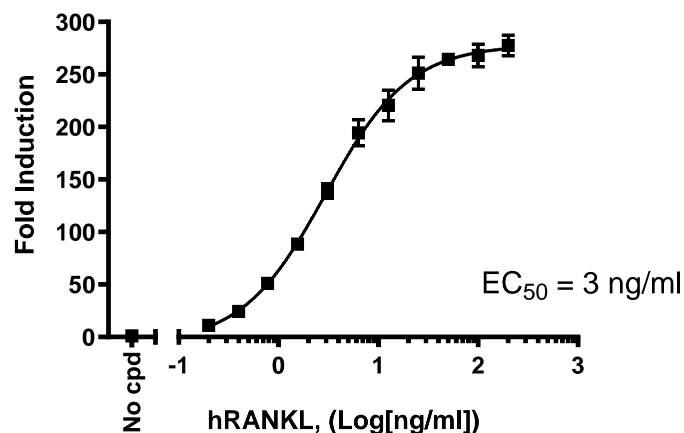


Figure 3. Dose response curve of RANK/NF-κB Luciferase Reporter HEK293 Cell Line to human RANKL.

Cells were treated with increasing concentrations of human RANKL for 6 hours. Luciferase activity was measured with ONE-Step™ luciferase reagent. Results are expressed as fold induction compared to the luciferase activity of unstimulated cells.

B. Inhibition of RANKL/RANK signaling in RANK/NF-κB Luciferase Reporter HEK293 Cell Line with an anti-RANK neutralizing antibody

1. Seed RANK/NF-κB Luciferase Reporter HEK293 cells in 90 µl of Thaw Medium 1 at a density of ~30,000 cells per well into a 96-well white clear-bottom cell culture plate. Leave a few wells empty to use as the "Cell-Free Control" (Background Signal).

2. Incubate the cells at 37°C in a CO_2 incubator for ~16 hours.

3. Prepare a 100 ng/ml solution of RANKL in Thaw Medium 1 (10 µl/well). This will be used in step 4.

For research use only. Not for human, drug, diagnostic, or therapeutic use.
BPS Bioscience
● bpsbioscience.com ● 858-202-1401 ● info@bpsbioscience.com

4. Prepare a three-fold serial dilution of Anti-RANKL Neutralizing Antibody, in the RANKL solution, at antibody concentrations 10-fold higher than the final desired concentrations (10 µl/well).

5. Add 10 µl of the anti-RANKL antibody dilution/RANKL mix to the “Treated” wells.

6. Add 10 µl of 100 ng/ml of RANKL solution to the "Untreated” wells.

7. Add 10 µl of Assay Medium to the “Unstimulated Control” wells.

8. Add 100 µl of Assay Medium to the “Cell-Free Control” (Background Signal) wells.

9. Incubate the plate at 37°C in a CO_2 incubator for 6 hours.

10. Add 100 µl of the ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 to 30 minutes.

11. Measure luminescence using a luminometer.

12. Data Analysis: Subtract average background luminescence (cell-free control wells) from the luminescence reading of all wells. The percent luminescence is the background-subtracted luminescence of the antibody-treated wells divided by the average background-subtracted luminescence of the untreated control wells x 100%.

$$Percent\ Luminescence = \left(\frac{Lumin.of\ antibody\ treated - background}{Lumin.of\ untreated - background}\right) x100$$

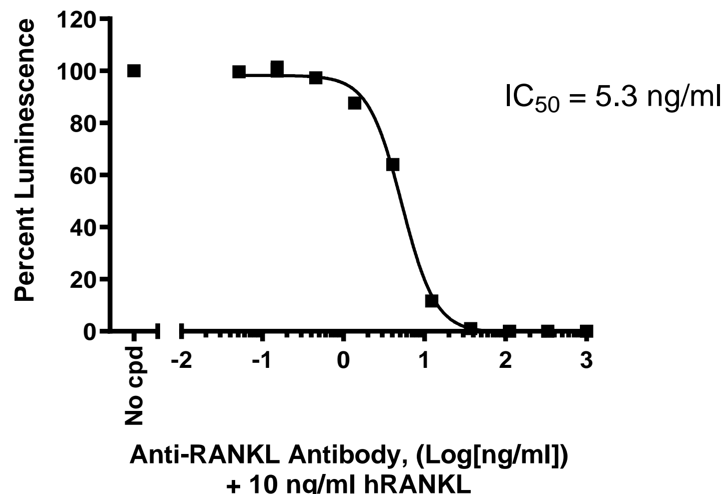


Figure 4. Inhibition of RANKL/RANK signaling by Anti-RANKL Neutralizing Antibody in RANK/NF-κB HEK293 Cell Line.
Cells were treated with RANKL in the presence or absence of Anti-RANKL Neutralizing Antibody for 6 hours. Luciferase activity was measured using ONE-Step™ luciferase reagent. Results are expressed as percent of control (in which luciferase activity in RANKL-stimulated cells without antibody was set at 100%).

For research use only. Not for human, drug, diagnostic, or therapeutic use.
● bpsbioscience.com ● 858-202-1401 ● info@bpsbioscience.com
BPS Bioscience

C. Inhibition of RANKL/RANK signaling in RANK/NF-κB Luciferase Reporter HEK293 Cell Line with a small molecule inhibitor

1. Seed RANK/NF-κB Luciferase Reporter HEK293 cells in 50 µl of Thaw Medium 1 at a density of ~30,000 cells per well into a 96-well white clear-bottom cell culture plate. Leave a few wells empty to use as the “Cell-Free Control” (Background Signal).

2. Incubate at 37°C with 5% CO_2 for 4-5 hours.

3. Prepare a serial dilution of inhibitor in Assay Medium at a concentration 2-fold higher than the final testing concentrations (50 µl/well).

4. Add 50 µl of diluted inhibitor to the “Treated” wells.

5. Add 50 µl of Assay Medium to the “Untreated” wells and “Unstimulated Control” wells.

6. Add 110 µl of Assay Medium to the “Cell-Free Control” (for determining background luminescence signal).

7. Incubate at 37°C with 5% CO_2 overnight (~16 hours).

8. The next day, prepare a solution of hRANKL in Assay Medium at a concentration 11-fold higher than the final desired concentration (10 µl/well). A final concentration of hRANKL of 10 ng/ml is suggested.

9. Add 10 µl of diluted RANKL to the “Treated” wells and to the “Untreated” wells.

10. Add 10 µl of Assay Medium to the “Unstimulated Control” wells.

11. Incubate at 37°C with 5% CO_2 for 5-6 hours.

12. Add 110 µl of ONE-Step™ Luciferase reagent per well.

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

14. Measure luminescence using a luminometer.

15. The “Background Control” luminescence value should be subtracted from all readings.

16. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The percent luminescence of luciferase reporter expression is the background-subtracted luminescence of treated wells divided by the average background-subtracted luminescence of the untreated control wells x 100%.

$$Percent\ Luminescence = \left(\frac{Luminescence\ of\ \ Treated\ Wells - avg.background}{Avg.Luminescence\ of\ Untreated\ Wells - avg.background}\right) x100$$

For research use only. Not for human, drug, diagnostic, or therapeutic use.
BPS Bioscience ● bpsbioscience.com ● 858-202-1401 ● info@bpsbioscience.com

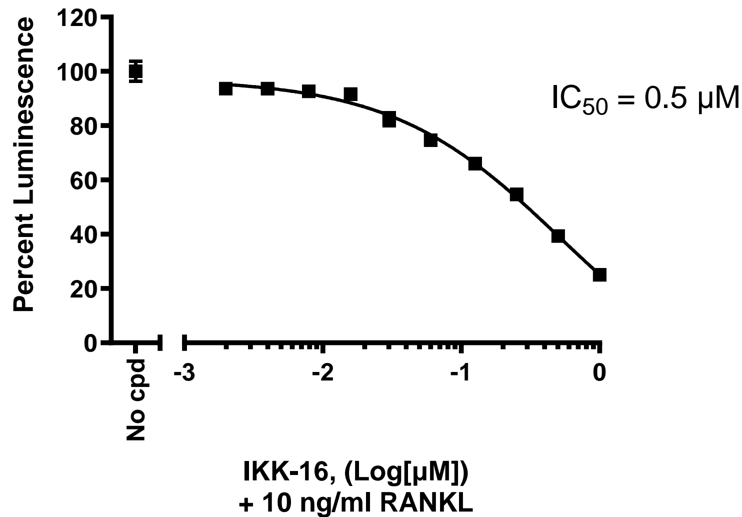


Figure 5. Inhibition of RANKL/RANK signaling by IKK-16 in RANK/NF-κB HEK293 Cell Line.
Cells were treated with increasing concentrations of IKK-16 dihydrochloride overnight before addition of RANKL to a final concentration of 10 mg/ml. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as percent luminescence of luciferase reporter activity (in which RANKL-stimulated cells in the absence of inhibitor is set to 100%).

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

Sequence
Human RANK sequence (accession number NM_003839.2)

MAPRARRRRPLFALLLLCALLARLQVALQIAPPCTSEKHYEHLGRCCNKCEPGKYMSSKCTTTSDSVCLPCGPDEYLDSWNEEDKC
LLHKVCDTGKALVAVVAGNSTTPRRCACTAGYHWSQDCECCRRNTECAPGLGAQHPLQLNKDTVCKPCLAGYFSDAFSSTDKC
RPWTNCTFLGKRVEHHGTEKSDAVCSSSLPARKPPNEPHVYLPGLIILLLFASVALVAAIIFGVCYRKKGKALTANLWHWINEACG
RLSGDKESSGDSCVSTHTANFGQQGACEGVLLLTLEEKTFPEDMCYPDQGGVCQGTCVGGGPYAQGEDARMLSLVSKTEIEEDS
FRQMPTEDEYMDRPSQPTDQLLFLTEPGSKSTPPFSEPLEVGENDSLSQCFTGTQSTVGSESCNCTEPLCRTDWTPMSSENYLQK
EVDSGHCPHWAASPSPNWADVCTGCRNPPGEDCEPLVGSPKRGPLPQCAYGMGLPPEEEASRTEARDQPEDGADGRLPSSAR
AGAGSGSSPGGQSPASGNVTGNSNSTFISSGQVMNFKGDIIVVYVSQTSQEGAAAAAEPMGRPVQEETLARRDSFAGNGPRFP
DPCGGPEGLREPEKASRPVQEQGGAKA

License Disclosure
Visit bpsbioscience.com/license for the label license and other key information about this product.

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

Related Products

Products	*Catalog #*	*Size*
Anti-RANKL Neutralizing Antibody	100874	50 µg
RANKL, His-Tag (Human)	71051	100 µg
RANK, Fc fusion (IgG1), Biotin Labeled (Human)	70822	25 µg
RANK, Fc fusion (IgG1), Avi-tag (Human)	70823	Various Sizes

Version 012524

BPS Bioscience

For research use only. Not for human, drug, diagnostic, or therapeutic use.

● bpsbioscience.com ● 858-202-1401 ● info@bpsbioscience.com