Description

Recombinant HEK-293 cells expressing firefly luciferase gene under the control of Nuclear factor-κB (NF-κB) with constitutive expression of human RANK (Receptor activator of nuclear factor-κB; TNFRSF11A; ref. seq. NM_003839.2).

Background

RANK/RANKL/OPG pathway plays a significant role in osteoclast maturation, which are the major cells in charge of bone resorption. If RANKL binds to the receptor RANK instead of binding to its decoy receptor Osteoprotegerin (OPG), it activates the NF-κB pathway to promote osteoclast survival and proliferation, resulting in the resorption of bone. Therefore, abnormal production of RANKL or OPG can cause osteoporosis and other bone-related disorders, making the RANKL/RANK signaling pathway a valuable target for drug development.

Application

Screen for inhibitors of RANKL/RANK signaling in a cellular context

Materials Provided

Components	Format
2 vials of frozen cells	2 x 10^6 cells in 1 ml of 10% DMSO

Host Cell

HEK293

Mycoplasma Testing

The cell line has been screened using the MycoAlert™ Mycoplasma Detection kit (Lonza, #LT07-218) 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 RANK	R&D Systems, #390-TN-010
RANKL antibody	BPS Bioscience #100874
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. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO2 using Growth Medium 1G with 5% CO2 using Growth Medium 1G.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium (Hyclone, #SH30024.01) supplemented with 10% FBS (Invitrogen, #26140-079), 1% non-essential amino acids (Hyclone, #SH30238.01), 1 mM Na pyruvate (Hyclone, #SH30239.01), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01)

Growth Medium 1G 3B (BPS Bioscience #79544):
Thaw Medium 1 (BPS Bioscience, #60187) plus 400 µg/ml of Geneticin (Life Technologies #11811031) and 50 µg/ml of Hygromycin B (Life Technologies, #10687-010)

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

Cell Culture Protocol

Cell Thawing

1. To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 1 (**no Geneticin and Hygromycin B**).
2. Spin down the cells, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 1 (**no Geneticin and Hygromycin B**).
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, add an additional ~3 ml of Thaw Medium 1 (**no Geneticin and Hygromycin B**), and continue growing culture in a CO2 incubator at 37°C until the cells are ready to be split.
5. Cells should be split before they are fully confluent. At first passage, switch to Growth Medium 1G (**contains Geneticin and Hygromycin B**).

Cell Passage

1. To passage the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA.
2. After detachment, add Growth Medium 1G (contains Geneticin and Hygromycin B) and transfer to a tube, spin down cells, resuspend cells in Growth Medium 1G and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ration: 1:6 weekly.

Cell Freezing

1. To freeze down the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA.
2. After detachment, add Thaw Medium 1 (**no Geneticin and Hygromycin B**) and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (BPS Bioscience #79796) at ~2 x 106 cells/ml.

3. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight.
4. Transfer to liquid nitrogen the next day for 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

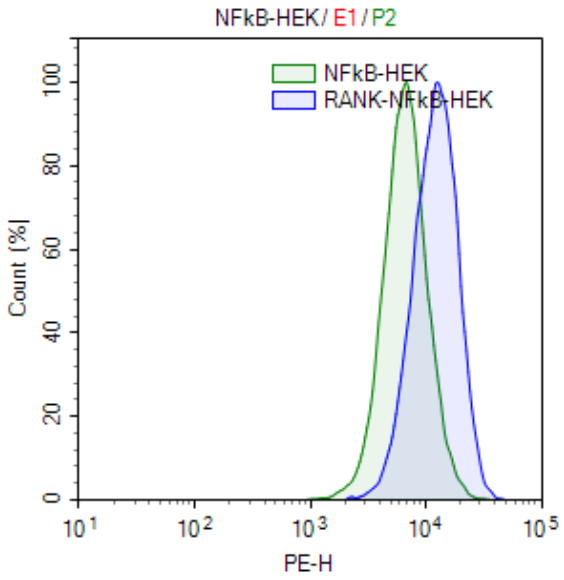


Figure 1.
RANK/NF-κB -HEK293 cells (blue) or control NF-κB -HEK293 cells (green) were stained with PE-labeled Anti-RANK Antibody (R&D systems, #FAB683P) and analyzed by FACS. Y-axis is the % cell number. X-axis is the intensity of PE.

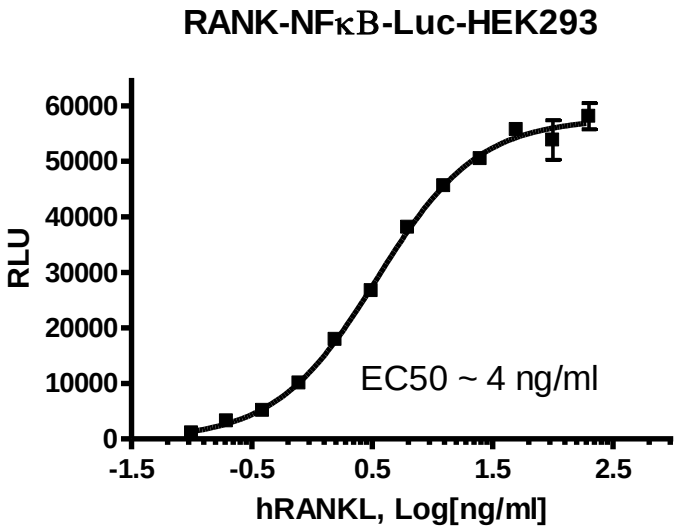
The following assays are designed for 96-well format. To perform the assay in a different format, the cell number and reagent volume should be scaled appropriately.

A. Dose response of RANK/NF-κB Reporter-HEK293 cells to human RANKL

1. Harvest RANK/NF-κB Reporter-HEK293 cells from culture in the Growth Medium 1G and seed cells at a density of ~30,000 cells per well into a white clear-bottom 96-well microplate in 90 µl of the Assay medium (see above). Leave a couple of wells empty for use as the cell-free control.
2. Incubate cells at 37°C in a CO_2 incubator for ~ 16 hours.
3. Add 10 µl of two-fold serial dilutions of human RANKL protein (R&D Systems, #390-TN 010) in Assay Medium to stimulated wells. Add 10 µl of assay medium to the unstimulated control wells. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate. Incubate the plate at 37°C in a CO_2 incubator for 6 hours.
4. Perform luciferase assay using ONE-Step Luciferase Assay buffer, according to the recommended instructions: Add 100 µl of the final ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 to 30 minutes. Measure luminescence using a luminometer.

5. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells.

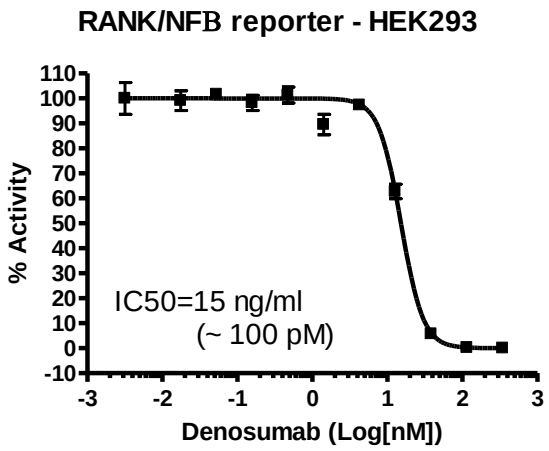
Figure 2.
Dose response of RANK/NF-κB Reporter -HEK293 cells to human RANKL



B. Inhibition of RANKL/RANK signaling in RANK/NF-κB Reporter-HEK293 cells

1. Harvest RANK/NF-κB Reporter-HEK293 cells from culture in the Growth Medium 1G and seed cells at a density of ~30,000 cells per well into a white clear-bottom 96-well microplate in 90 µl of the Assay Medium. Leave a couple of wells empty as a cell-free control.
2. Incubate cells at 37°C in a CO_2 incubator for ~ 16 hours.
3. Prepare 100 µl of serially diluted anti-RANKL antibody (BPS Bioscience #100874) plus RANKL (at EC75 ~ EC85 concentration) solution in the Assay Medium.
4. Add 10 µl of the anti-RANKL antibody/RANKL mixture to the cells and incubate the plate at 37°C in a CO_2 incubator for 6 hours.
5. Perform luciferase assay using ONE-Step™ Luciferase Assay kit, according to the recommended instructions: Add 100 µl of the final ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 to 30 minutes. Measure luminescence using a luminometer.
6. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells.

Figure 3.
Inhibition of RANKL/RANK signaling by anti-RANKL antibody (Denosumab) in RANK/NF-κB -HEK293 cells



Sequence
Human RANK sequence (accession number NM_003839.2)

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LLHKVCDTGKALVAVVAGNSTTPRRCACTAGYHWSQDCECCRRNTECAPGLGAQHPLQLNKDTVCKPCLAGYFSDAFSSTDKC
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AGAGSGSSPGGQSPASGNVTGNSNSTFISSGQVMNFKGDIIVVYVSQTSQEGAAAAAEPMGRPVQEETLARRDSFAGNGPRFP
DPCGGPEGLREPEKASRPVQEQGGAKA

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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*
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