HEK-Blue™ RANKL Cells

Human & Murine RANKL Reporter Cells

Catalog code: hkb-rankl

https://www.invivogen.com/hek-blue-rankl

For research use only

Version 19G11-NJ

PRODUCT INFORMATION

Contents

- **1 vial of HEK-Blue™ RANKL cells** (3-7 x 10^6 cells)
- **1 ml of Normocin™** (50 mg/ml), a formulation of three antibiotics active against mycoplasmas, bacteria, and fungi. Store at -20 °C.*
- **1 ml of Blasticidin (10 mg/ml).** Store at 4°C or at -20°C.*
- **1 ml of Zeocin™ (100 mg/ml).** Store at 4°C or at -20°C.*

*The expiry date is specified on the product label.

- **1 ml of QB reagent** and **1 ml of QB buffer** (sufficient to prepare 100 ml of **QUANTI-Blue™ Solution**, a SEAP detection reagent). QB reagent and QB buffer are stable for 1 year at -20 °C. QUANTI-Blue™ Solution is stable for 2 weeks at 4 °C and for 2 months at -20 °C.

Handling Cells Upon Arrival

Cells must be thawed immediately upon receipt and grown according to handling procedures (as described on the next page) to ensure the best cell viability and proper assay performance.

*Note: **Avoid freezing cells upon receipt** as it may result in irreversible damage to the cell line.*

Disclaimer: We cannot guarantee cell viability if the cells are not thawed immediately upon receipt and grown according to handling procedures.

Cell Line Stability

Cells will undergo genotypic changes over time that will result in reduced responsiveness in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages.

Quality Control

- SEAP reporter activity in response to RANKL and various cytokines has been validated using functional assays.
- The stability for 20 passages, following thawing, has been verified.
- These cells are guaranteed mycoplasma-free.

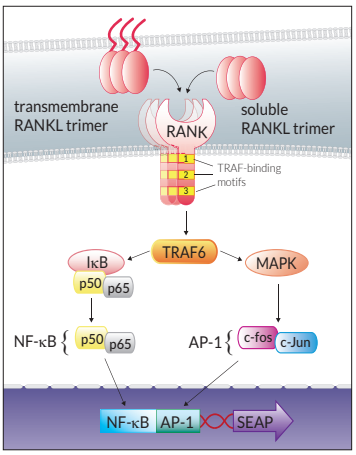
USE RESTRICTIONS

These cells are distributed for research purposes only.

This product is covered by a Limited Use License. By use of this product, the buyer agrees with the terms and conditions of all applicable Limited Use Label Licenses.

For non-research use, such as screening, quality control or clinical development, contact info@invivogen.com.

BACKGROUND



Receptor Activator of NF-κB Ligand (RANKL), also known as member 11 of the tumor necrosis factor (TNF) superfamily (TNFSF11) or TNF-related activation-induced cytokine (TRANCE), exists as a transmembrane or soluble protein produced by osteoblasts and activated T cells[1]. RANKL binds to its receptor RANK via an obligate trimer configuration[1,2]. RANKL/RANK signaling plays a pivotal role in bone remodeling and dendritic cell survival, thereby enhancing induction of T cell responses[1]. Upon RANKL binding, RANK trimers recruit TNF receptor-associated factor (TRAF) adaptor proteins, such as TRAF6, to TRAF-binding motifs within their cytoplasmic domains[1]. The TRAF6 signaling cascade results in the activation of NF-κB and AP-1 transcription factors. Multiple efforts have focused on the development of anti-RANKL antibodies or small-molecule inhibitors for blocking RANKL/RANK signaling to reduce osteoporosis, prevent skeletal-related events (SREs) from bone metastasis in cancer, or improve anti-tumor immunity[1-3].

1. Cheng ML. & Fong L., 2014. Effects of RANKL-targeted therapy in immunity and cancer. Front. Oncol. 3:329. **2. Ahern E. *et al.*, 2018.** Roles of the RANKL-RANK axis in anti-tumour immunity — implications for therapy. Nat. Rev. Clin. Oncol. 15:676-93. **3. Nakai Y. *et al.*, 2019.** Efficacy of an orally active small-molecule inhibitor of RANKL in bone metastasis. Bone Res. 7:1.

PRODUCT DESCRIPTION

HEK-Blue™ RANKL cells were designed for the detection of bioactive human RANKL (hRANKL) and murine RANKL (mRANKL) by monitoring the activation of the NF-κB and AP-1 pathways. These cells can be used for screening antibodies or inhibitors targeting the RANKL/RANK pathway. HEK-Blue™ RANKL cells were generated by stable transfection of the human embryonic kidney HEK293 cell line with the genes encoding hRANK and an NF-κB/AP1-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. Binding of RANKL to its receptor on the surface of HEK-Blue™ RANKL cells triggers TRAF6-mediated activation of NF-κB/AP1 and the subsequent production of SEAP. This can be readily assessed in the culture supernatant using QUANTI-Blue™ Solution, a SEAP detection reagent. HEK-Blue™ RANKL cells are resistant to blasticidin and Zeocin™.

Detection range for hRANKL: 3-100 ng/ml
Detection range for mRANKL: 1-100 ng/ml

TECHNICAL SUPPORT
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FAQ Any questions about our cell lines?
Visit our FAQ page.

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SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ RANKL cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) that require **Biosafety level 2** according to the American Center for Disease Control and Prevention (CDC) guidelines. The biosafety level may vary depending on the country. For example, in Germany HEK293 cell lines are designated Biosafety Level 1 according to the Central Committee of Biological Safety, Zentrale Kommission für die Biologische Sicherheit (ZKBS). Please check with your country's regulatory authority regarding the use of these cells.

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum (FBS; 30 min at 56 °C), 100 µg/ml Normocin™, Pen-Strep (100 U/ml-100 µg/ml)
- **Freezing Medium:** DMEM, 4.5 g/l glucose, 20% FBS, 10% DMSO
- **Test Medium:** DMEM 4.5 g/l glucose, 2 mM L-glutamine, 10% heat-inactivated FBS, Pen-Strep (100 U/ml-100 µg/ml) **without Normocin™, Blasticidin and Zeocin™**

Required Selection Antibiotics

- Blasticidin and Zeocin™

Initial Culture Procedure

The first propagation of cells should be for generating stocks for future use. This ensures the stability and performance of the cells for subsequent experiments.

1. Thaw the vial by gentle agitation in a 37 °C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid.
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% (v/v) ethanol.

Note: All steps from this point should be carried out under strict aseptic conditions.

3. Transfer cells to a larger tube containing 15 ml of pre-warmed growth medium. **Do not add Blasticidin and Zeocin™ until the cells have been passaged twice.**
4. Centrifuge at 1000-1200 RPM (RCF 200-300 g) for 5 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selection antibiotics.
6. Transfer the cells to a T-25 culture flask containing 5 ml of growth medium.
7. Place the culture at 37 °C in 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10^6 cells/ml in freshly prepared freezing medium.

Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.

2. Prepare 1 ml aliquots of cells in cryogenic vials.
3. Place vials in a freezing container and store at -80 °C overnight.
4. Transfer vials to liquid nitrogen for long term storage.

Note: If properly stored, cells should remain stable for years.

Cell Maintenance

1. Maintain and subculture the cells in growth medium supplemented with 30 µg/ml of blasticidin and 100 µg/ml of Zeocin™.
2. Renew the growth medium twice a week.
3. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

Cell Handling Recommendations

To ensure the best results:

- Use HEK-Blue™ RANKL cells with less than 20 passages.

REPORTER ASSAY

Day 1:

1. Prepare HEK-Blue™ RANKL cell suspension: gently rinse cells twice with pre-warmed phosphate buffered saline (PBS) and then detach the cells in the presence of PBS by tapping the flask. Resuspend cells in fresh, pre-warmed test medium and prepare a cell suspension at ~280,000 cells/ml.

*Note: The response of HEK-Blue™ RANKL cells can be altered by the action of trypsin. Do **not** use trypsin to detach HEK-Blue™ RANKL cells.*

2. Add 20 µl of each sample per well of a flat-bottom 96-well plate.
3. Add 20 µl of recombinant human or murine RANKL at 10 ng/ml (positive control) in one well.
4. Add 20 µl of a recombinant cytokine such as recombinant human TNF-α at 10 ng/ml (negative control) in one well.
5. Add 180 µl of cell suspension (~50,000 cells) per well.
6. Incubate the plate at 37 °C in a CO_2 incubator for 20-24 h.

Day 2:

1. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed data sheet.
2. Add 20 µl of induced HEK-Blue™ RANKL cell supernatant per well of a flat-bottom 96-well plate.
3. Add 180 µl of resuspended QUANTI-Blue™ Solution per well.
4. Incubate the plate at 37 °C for 1-3 h.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Description	Cat. Code
Anti-hRANKL-hIgA2	Monoclonal antibody	hrankl-mab7
Anti-hRANKL-hIgG1	Monoclonal antibody	hrankl-mab1
Anti-hRANKL-hIgG2	Monoclonal antibody	hrankl-mab2
Blasticidin	Selection antibiotic	ant-bl-1
Normocin™	Antimicrobial Reagent	ant-nr-1
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
Recombinant human TNF-α	Recombinant cytokine	rcyc-htnfa
Zeocin™	Selection antibiotic	ant-zn-1