

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 23B27-MM

PRODUCT INFORMATION

Contents

• 3-7 x 10⁶ of HEK-Blue™ RANKL cells in a cryovial or shipping flask. **IMPORTANT:** If cells provided in a cryovial are not frozen upon arrival, contact InvivoGen immediately.

• 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.

Note: Data sheets for all components are available on our website.

Handling Frozen 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.

IMPORTANT: For cells that arrive in a shipping flask please refer to the enclosed 'cell recovery procedure'.

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

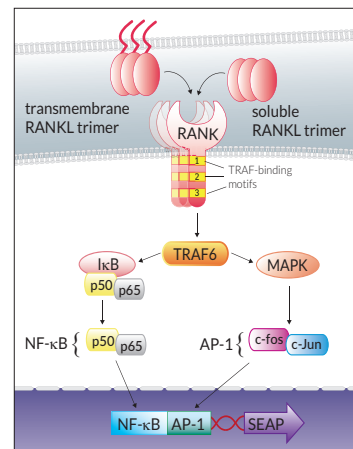
These cells are distributed for research purposes only.

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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 cells¹. 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 responses¹. Upon RANKL binding, RANK trimers recruit TNF receptor-associated factor (TRAF) adaptor proteins, such as TRAF6, to TRAF-binding motifs within their cytoplasmic domains¹. 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 immunity¹⁻³.



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-tumor 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

InvivoGen USA (Toll-Free): 888-457-5873

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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™, Blastidicin and Zeocin™**

Required Selection Antibiotics

- **Blastidicin** 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 Blastidicin and Zeocin™ until the cells have been passaged twice.**
4. Centrifuge at 150 x g (RCF) for 10 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% CO₂.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10⁶ 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. HEK-Blue™ RANKL cells grow as adherent cells. Detach the cells using trypsin for 2-3 min at room temperature (RT).
Note: Prolonged action of trypsin or incubation at 37°C may alter the cell surface expression of receptors.
2. Maintain and subculture the cells in growth medium supplemented with 30 µg/ml of **blastidicin** and 100 µg/ml of **Zeocin™**.
3. Renew the growth medium twice a week.
4. 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), detach the cells in the presence of PBS for 2-3 min at 37°C. Tap the flask if needed. Resuspend cells in fresh, pre-warmed test medium and prepare a cell suspension at ~280,000 cells/ml.

Note: We recommend avoiding the use of trypsin to detach cells for the functional assays (see FAQs online).

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 CO₂ 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-hlgA2	Monoclonal antibody	hrankl-mab7
Anti-hRANKL-hlgG1	Monoclonal antibody	hrankl-mab1
Anti-hRANKL-hlgG2	Monoclonal antibody	hrankl-mab2
Blastidicin	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

TECHNICAL SUPPORT

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QUANTI-Blue™ Solution

Medium for detection and quantification of alkaline phosphatase in standard and HTS assays

Catalog code: rep-qbs, rep-qbs2, rep-qbs3

<https://www.invivogen.com/ quanti-blue>

For research use only

Version 23A12-MM

PRODUCT INFORMATION

Contents: QUANTI-Blue™ Solution is available in three pack sizes

- **rep-qbs:** 5 x 1 ml of QB reagent and 5 x 1 ml QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **25 x 96-well plates** (500 ml using the standard procedure) or **20 x 1536-well plates** (85 ml using the HTS screening procedure).

- **rep-qbs2:** 10 x 1 ml of QB reagent and 10 x 1 ml QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **50 x 96-well plates** (1 L using the standard procedure) or **40 x 1536-well plates** (170 ml using the HTS screening procedure).

- **rep-qbs3:** 1 x 20 ml bottle of QB reagent and 1 x 20 ml bottle of QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **100 x 96-well plates** (2 L using the standard procedure) or **80 x 1536-well plates** (340 ml using the HTS screening procedure).

Required Material (not provided)

- Sterile water
- Sterile screw cap tube, glass bottle or flask

Storage and stability

- Product is shipped at room temperature. Upon receipt, store QB reagent and QB buffer at -20°C. Product is stable for 1 year at -20°C when properly stored.

- The 20 ml bottles of QB reagent and QB buffer are designed for single use. If required, individual aliquots of QB reagent and QB buffer can be prepared upon receipt or following a single freeze-thaw cycle. Store aliquots at -20°C. **Avoid repeated freeze-thaw cycles.**

Note: During storage, a precipitate may form in the 20 ml bottle of QB reagent. If this occurs, vortex the product until the precipitate disappears. The formation of a precipitate does not affect the activity of the product.

- Reconstituted QUANTI-Blue™ Solution is stable for 2 weeks at 2-8°C and for 2 months at -20°C. Protect QUANTI-Blue™ from light.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.

- Physicochemical characterization (pH, appearance).
- Functional assays using alkaline phosphatase or SEAP-expressing reporter cells.

DESCRIPTION

QUANTI-Blue™ is a colorimetric enzyme assay developed to determine any alkaline phosphatase activity (AP) in a biological sample, such as supernatants of cell cultures. QUANTI-Blue™ Solution changes from pink to a purple-blue color in the presence of AP. Secreted embryonic alkaline phosphatase (SEAP) is a widely used reporter gene. It is a truncated form of placental alkaline phosphatase, a glycosylphosphatidylinositol (GPI)-anchored protein. SEAP is secreted into the cell culture supernatant and therefore offers many advantages over intracellular reporters.

QUANTI-Blue™ is highly sensitive for quantitative measurement. It has a higher saturation threshold than with pNPP (p-nitrophenyl phosphate) resulting in more significant differences between no, low or high AP activity. Another advantage of QUANTI-Blue™ is that it can determine secreted AP activity without disturbing cells, thus allowing the repeated sampling of cell cultures for kinetic studies.

TECHNICAL SUPPORT

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METHODS

QUANTI-Blue™ Solution has been optimized for use in 96-well plates (standard procedure) and in 1536-well plates (high throughput screening procedure).

A. Standard procedure

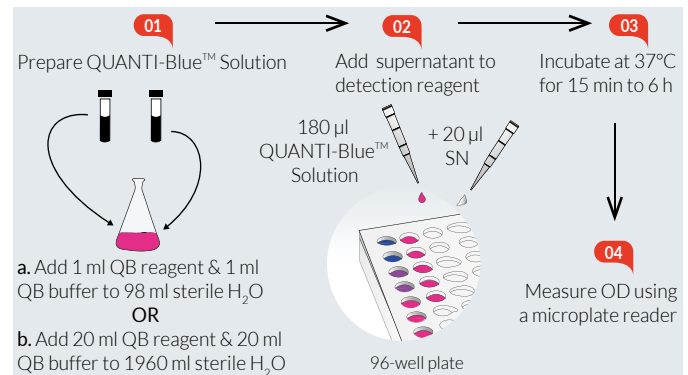


Figure 1. Standard procedure using QUANTI-Blue™ Solution.

The following protocol refers to the use of 96-well plates. Ensure QB reagent and QB buffer are completely thawed before use.

Note: For fast thawing, QB reagent and QB buffer can be placed at 37°C for 2 minutes. Ensure heating at 37°C does **not** exceed 5 minutes.

1. In a sterile bottle or flask, prepare QUANTI-Blue™ Solution by adding:
 - a. 1 ml of QB reagent and 1 ml of QB buffer to 98 ml of sterile water.
 - b. 20 ml of QB reagent and 20 ml of QB buffer to 1960 ml of sterile water.
2. Mix by vortexing and incubate at room temperature for 10 min before use.
3. Use QUANTI-Blue™ Solution immediately or store at 2-8°C or -20°C.
4. Dispense 180 µl of QUANTI-Blue™ Solution per well into a flat-bottom 96-well plate.
5. Add 20 µl of the sample (supernatant of SEAP-expressing cells) or negative control (cell culture medium).
6. Incubate at 37°C for 15 min to 6 h.
7. Measure optical density (OD) at 620-655 nm using a microplate reader.

Note: If the negative control turns purple/blue, it means the fetal bovine serum (FBS) contains alkaline phosphatase. We recommend heating FBS at 56°C for 30 min to inactivate the alkaline phosphatase activity.

For different cell culture plate formats, please refer to the table below:

	96-well plate	24-well plate	12-well plate
QUANTI-Blue™	180 µl	450 µl	900 µl
Supernatant	20 µl	50 µl	100 µl

B. High Throughput Screening (HTS) procedure

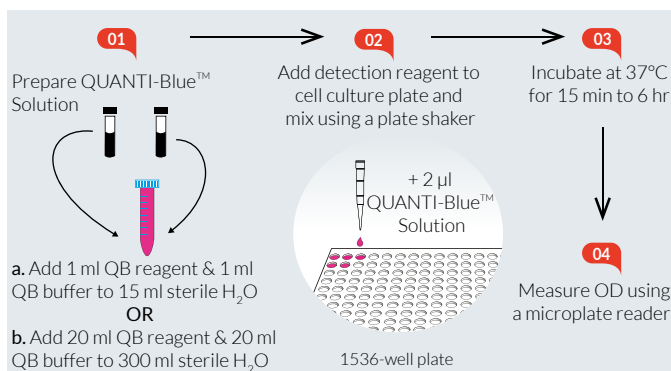


Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

This procedure has been optimized for use in HTS screening procedures in 1536-well plates. QUANTI-Blue™ Solution is added directly to the cell suspension to reduce liquid handling.

Ensure QB reagent and QB buffer are completely thawed before use. *Note:* For fast thawing, QB reagent and QB buffer can be placed at 37°C for 2 minutes. Ensure heating at 37°C does **not** exceed 5 minutes.

1. Dispense cell suspension and test compounds into a 1536-well plate in a volume that does not exceed **5 µl** per well. Incubate cells with test compounds for the desired period of time.
2. Prepare QUANTI-Blue™ Solution by adding:
 - a. **1 ml** of QB reagent and **1 ml** of QB buffer to **15 ml** of sterile water in a sterile 50 ml screw cap tube.
 - b. **20 ml** of QB reagent and **20 ml** of QB buffer to **300 ml** of sterile water in a sterile glass bottle or flask.
3. Mix well by vortexing and incubate at room temperature for 10 minutes before use.
4. Use QUANTI-Blue™ Solution immediately or store at 2-8°C or -20°C.
5. Dispense **2 µl** of QUANTI-Blue™ Solution to the wells containing $\leq 5 \mu\text{l}$ of cell culture in a 1536-well plate.
6. Mix using a plate shaker.
7. Incubate at 37°C for 15 min to 6 h.
8. Measure OD at 620-655 nm.

Note: If the negative control turns purple/blue, it means the fetal bovine serum (FBS) contains alkaline phosphatase. We recommend heating FBS at 56°C for 30 min to inactivate the alkaline phosphatase activity.

RELATED PRODUCTS

Product	Catalog Code
pNifTy2-SEAP (Zeo®)	pnifty2-seap
pSELECT-zeo-SEAP	psetz-seap
HEK-Blue™ Detection	hb-det2
Recombinant SEAP Protein	rec-hseap
Reporter cells	
HEK-Blue™ hTLR2	hkb-htlr2
HEK-Blue™ hTLR4	hkb-htlr4
RAW-Blue™ Cells	raw-sp
THP1-Blue™ NF-κB Cells	thp-nfkb
THP1-Blue™ ISG Cells	thp-isg

For a complete list of InvivoGen's Reporter Cell Lines visit <https://www.invivogen.com/reporter-cells>

TECHNICAL SUPPORT

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