Ramos-Blue™ Cells

NF-κB/AP-1 Reporter B lymphocytes

Catalog code: rms-sp

https://www.invivogen.com/ramos-blue-cell

For research use only

Version 19E08-MM

PRODUCT INFORMATION

Contents and Storage

- **1 vial of Ramos-Blue™ cells** (3-7 x 10^6 cells) in freezing medium

IMPORTANT: Cells are shipped frozen. If cells are not frozen upon arrival, contact InvivoGen immediately.

- **1 ml of Zeocin™** (100 mg/ml). Store Zeocin™ at 4 °C or at -20 °C.*
- **1 ml of Normocin™** (50 mg/ml). Normocin™ is a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store 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 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 resulting in reduced responsiveness over time 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

- Reporter activity has been validated using functional assays.
- Expression of TLRs and NOD1 has been determined by RT-PCR.
- Stability for 20 passages following thawing has been verified.
- The cell line is guaranteed mycoplasma-free.

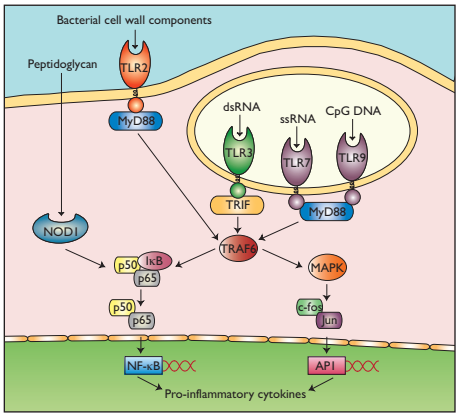
RESTRICTIONS

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 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.

INTRODUCTION

B lymphocytes are key players of the adaptive immune system but are also prominent in the innate immune response. Consistent with their dual role, they express Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs) that allow them to discriminate among a wide spectrum of pathogen-associated molecules (PAMPs). Upon PRR stimulation by PAMPs, various signaling pathways are induced leading to the activation of transcription factors, such as NF-κB and AP-1, and the subsequent production of inflammatory cytokines.



PRODUCT DESCRIPTION

Ramos-Blue™ is a B lymphocyte cell line that stably expresses an NF-κB/AP-1-inducible SEAP (secreted embryonic alkaline phosphate) reporter gene. Ramos-Blue™ cells derive from a human Burkitt's lymphoma which is negative for Epstein Barr virus. They have the characteristics of B lymphocytes and are routinely used as a model of B lymphocytes and for apoptosis studies. The Ramos-Blue™ cell line was isolated for its ability to respond to CpG ODNs (TLR9 ligands).

Ramos-Blue™ cells are responsive to NF-κB inducers, such as TNF-α, TLR3, TLR7, TLR9 and NOD1 agonists. When stimulated, they produce SEAP in the supernatant that can be readily monitored using the QUANTI-Blue™ assay. QUANTI-Blue™ is a SEAP detection medium that turns blue in the presence of SEAP. Levels of SEAP can be observed with the naked eye or measured using a spectrophotometer at 620-655 nm.

Ramos-Blue™ cells are resistant to Zeocin™.

APPLICATIONS

The Ramos-Blue cell line can be used to study the NF-κB and AP-1 signaling pathways in B lymphocytes and in particular the TLR3, TLR7, TLR9 and NOD1 signaling pathways.

TECHNICAL SUPPORT
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SAFETY CONSIDERATIONS

Biosafety Level 1

HANDLING PROCEDURES

Required Cell Culture Medium

• **Growth Medium:** IMDM (Iscove's Modified Dulbecco's Medium), 2 mM L-glutamine, 10% v/v heat-inactivated fetal bovine serum (30 min at 56 °C; FBS), 100 µg/ml Normocin™, Pen-Strep (100 U/ml-100 µg/ml).
Notes: The use of Normocin™ together with Pen-Strep is required to keep the cells free of microbial contaminants.
• **Freezing Medium:** IMDM, 20% v/v FBS, 10% v/v DMSO

Required Selective Antibiotic

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% ethanol.
Note: All steps from this point should be carried out under aseptic conditions.
3. Transfer cells in a larger vial containing 15 ml of pre-warmed growth medium.
4. Centrifuge vial at 300 g for 5 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium. **Do not add selective antibiotic.**
6. Transfer the vial contents 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 freezing medium prepared extemporaneously with cold growth medium.
2. Aliquot 1 ml cells into 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 the cells in growth medium supplemented with 100 µg/ml Zeocin™.
2. Pass the cells every 3-4 days at a density of at least 0.5 x 10^6 cells/ml.
Notes: To ensure the best results
- Cell density should not exceed 6 x 10^6 cells/ml.
- Use Ramos-Blue™ cells with less than 20 passages.

QUANTI-BLUE™ ASSAY

The QUANTI-Blue™ assay allows to detect NF-kB/AP-1 activation in Ramos-Blue™ cells stimulated by molecules such as TNF-α or poly(I:C).

Stimulation of Ramos-Blue™ cells

1. Rinse cells with fresh growth medium without selective antibiotics.
2. Count cells and resuspend cells in fresh growth medium without selective antibiotics at a density of 2 x 10^6 cells/ml.
3. Add 20 µl of an NF-kB/AP-1 activator at various concentrations per well of a flat-bottom 96-well plate including a negative control, such as endotoxin-free water.
4. Add 180 µl of the cell suspension (~400,000 cells) per well.
IMPORTANT: To ensure reliable and reproducible results, homogenize the cell suspension using a pipette.
5. Incubate the plate at 37 °C in a 5% CO_2 incubator for 18-24 h.

Detection of NF-κB/AP-1 activation

1. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed product data sheet.
2. Add 40 µl of supernatant from stimulated Ramos-Blue™ cells .
3. Add 160 µl of QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
4. Incubate the plate at 37 °C for 30 min to 6 h.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

CELL LINE VALIDATION

Ramos-Blue™ cells express all TLRs and NOD1 as detected by RT-PCR (figure 1). However, activation of NF-κB/AP-1 was only observed following stimulation with TLR2, TLR3, TLR7, TLR9 and NOD1 agonists (figures 2 and 3).

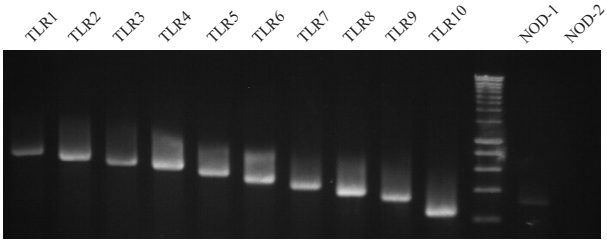

Figure 1: Expression of TLR and NOD mRNAs in Ramos-Blue™ cells determined by RT-PCR using primers specific for human TLR and NOD.

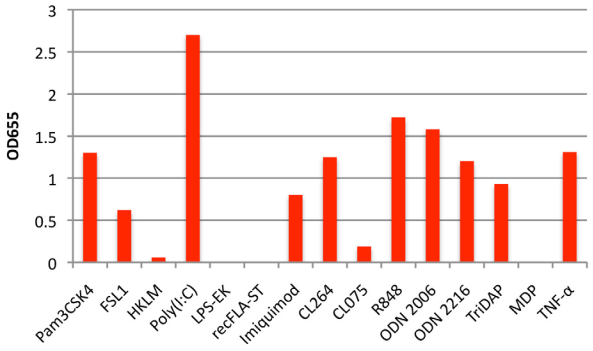

Figure 2: NF-κB/AP-1 activation in Ramos-Blue™ cells induced by various activators. Cells were incubated with 10 µg/ml Pam3CSK4 (TLR1/2), FSL-1 (TLR2/6), HKLM (TLR2, 1x10^9 cells/ml), poly(I:C) HMW(TLR3), LPS-EK Ultrapure (TLR4), recFLA-ST (TLR5), imiquimod and CL264 (TLR7), CL075 (TLR8), R848 (TLR7/8), ODN2006 and ODN2216 (TLR9), TriDAP (NOD1), MDP (NOD2) and TNF-a. After 24h incubation, NF-kB/AP-1 activation was determined using QUANTI-Blue™.

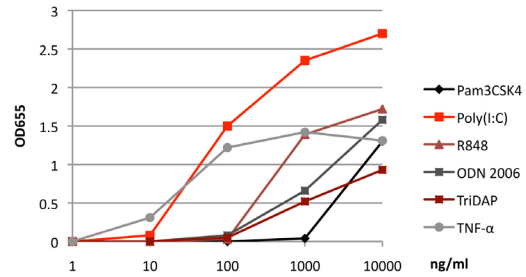

Figure 3: Dose responses to various activators. Ramos™-Blue cells were incubated with increasing concentrations of Pam3CSK4, poly(I:C) HMW, R848, ODN2006, TriDAP and TNF-a. After 24h incubation, NF-κB/AP-1 activation was determined using QUANTI-Blue™.

RELATED PRODUCTS

Product	Description	Cat. Code
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs
Ramos-Blue™ KD-MyD Cells	MyD88 knockdown cells	rms-kdmyd
Zeocin™	Selective 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

http://www.invivogen.com/quanti-blue

For research use only

Version 18D13-MM

PRODUCT INFORMATION

Contents

QUANTI-Blue™ Solution is available in two pack sizes:

- **rep-qbs** containing 5 x 1 ml of QB reagent and 5 x 1 ml QB buffer to prepare 500 ml of QUANTI-Blue™ Solution sufficient for 25 x 96-well plates (standard procedure) or 20 x 1536-well plates (HTS screening)
- **rep-qbs2** containing 10 x 1 ml of QB reagent and 10 x 1 ml QB buffer to prepare 1 liter of QUANTI-Blue™ Solution sufficient for 50 x 96-well plates (standard procedure) or 40 x 1536-well plates (HTS screening)

Required Material (not provided)

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

Storage and Stability

- Store QB reagent and QB buffer at -20 °C. Product is stable for 1 year at -20 °C when properly stored.
- Reconstituted QUANTI-Blue™ Solution is stable for 2 weeks at 2-8 °C and for 2 months at -20 °C. Keep reconstituted QUANTI-Blue™ away from light.

Quality Control

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

- Physicochemical characterization (including pH, solubility).
- 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 GPI-anchored protein. SEAP is secreted into cell culture supernatant and therefore offers many advantages over intracellular reporters.

FEATURES AND ADVANTAGES

- **Requires small samples of cell supernatants** - 20 µl is sufficient.
- **No need to process samples** - Preparation of cell lysates or heating of samples is not required.
- **Determine secreted AP activity without disturbing cells** - The same cell cultures can be repeatedly sampled for kinetic studies.
- **Assay can be completed in 30 min** - Hands-on time no longer than 10 min. The enzymatic activity can be detected as early as 15 min after incubation of the samples in QUANTI-Blue™ Solution.
- **Wide dynamic range allows to detect low and high levels of AP** - No need to perform multiple sample dilutions.
- **Highly sensitive for quantitative measurement** - Higher saturation threshold than with pNPP (p-nitrophenyl phosphate) resulting in more significant differences between no, low or high AP activity.
- **Extremely simple to use** - 1) Prepare solution with water, 2) add sample to detection reagent, 3) incubate at 37 °C, and 4) assess AP activity.

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

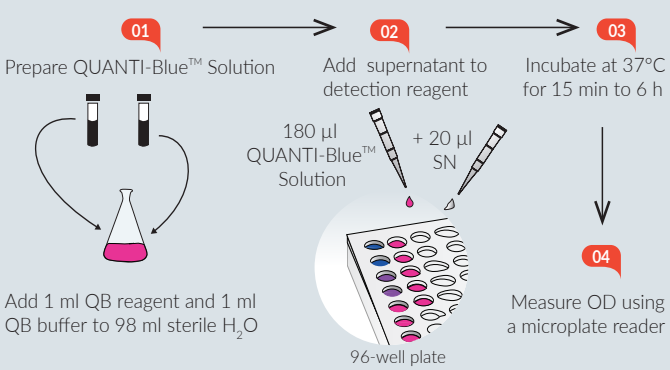

Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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. Prepare 100 ml of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml of QB buffer to 98 ml of sterile water in a sterile glass bottle or flask.
2. Mix well 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 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 to heat 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

TECHNICAL SUPPORT
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B. High Throughput Screening procedure

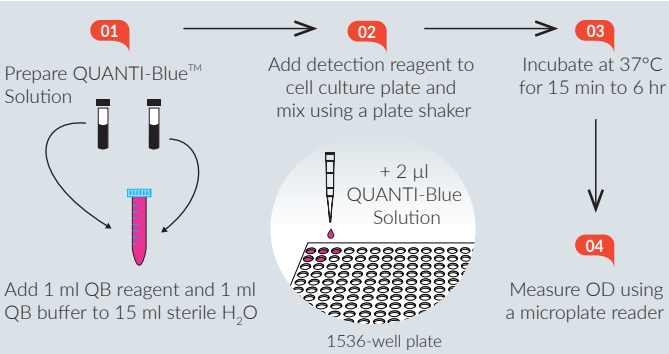

Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

This procedure has been optimized for use directly in flat-bottom 1536-well plates, in which cell culture volume does not exceed **5 µl**. 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. Prepare 17 ml of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml of QB buffer to 15 ml of sterile water in a 50 ml screw cap tube.
2. Mix well by vortexing and incubate at room temperature for 10 minutes before use.
3. Use QUANTI-Blue™ Solution immediately or store at 2-8 °C or -20 °C.
4. Dispense 2 µl of QUANTI-Blue™ Solution per well of a 1536-well plate.
5. Mix using a plate shaker.
6. Incubate at 37 °C for 15 min to 6 h.
7. Measure 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 to heat FBS at 56 °C for 30 min to inactivate the alkaline phosphatase activity.

RELATED PRODUCTS

Product	Catalog Code
pNiFty2-SEAP (Zeo^R)	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
http://www.invivogen.com/reporter-cells