RAW-Blue™ Cells
Mouse Macrophage Reporter Cell Line
Catalog code: raw-sp
https://www.invivogen.com/raw-blue
For research use only
Version 19K14-MM

PRODUCT INFORMATION
Contents and Storage
- 1 vial of RAW-Blue™ cells (3.7 x 10⁶ cells)

IMPORTANT: Cells are shipped frozen in Europe, USA & Canada. If cells are not frozen upon arrival, contact InvivoGen immediately.
- 1 ml of Zeocin™ (100 mg/ml). Store at 4°C or at -20°C.
- 1 ml of Normocin™ (50 mg/ml), 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
- Expression of TLRs (TLR1 to TLR9), RLRs (RIG-I and MDA-5), NODs (NOD1 and NOD2) and Dectin-1 was determined by RT-PCR (figure 1).
- Stimulation of TLRs, NODs and Dectin-1 by pathogen-associated molecular patterns was assessed using the QUANTI-Blue™ assay (see figures 2A and 2B).
- The cells are guaranteed mycoplasma-free.

RAW-Blue” Cells are derived from RAW 264.7 macrophages. They stably express a secreted embryonic alkaline phosphatase (SEAP) gene inducible by NF-κB and AP-1 transcription factors. RAW-Blue™ Cells express all TLRs (with the exception of TLR5) as well as RIG-I, MDA-5, NOD1 and NOD2; expression of TLR3 and NOD1 being very low. The presence of specific agonists of these receptors induces signaling pathways leading to the activation of NF-κB and AP-1. RAW-Blue™ cells can also be used as a Dectin-1 reporter cell line as they express high levels of endogenous Dectin-1. Stimulation of RAW-Blue™ cells with zymosan or heat-killed preparations of yeast induces the activation of NF-κB in a Dectin-1-dependent manner. Upon TLR, NOD or Dectin-1 stimulation, RAW-Blue™ cells activate NF-κB and/or AP-1 leading to the secretion of SEAP which is easily detectable and measurable when using QUANTI-Blue™, SEAP detection medium.

RAW-Blue™ Cells are resistant to Zeocin™ and G418. Cells should be maintained in growth medium (described on the next page) supplemented with Zeocin® only. Antibiotic pressure with Zeocin® is required to maintain the plasmid coding for SEAP.

Figure 1: Expression of TLR, RLR, NOD and Dectin-1 mRNAs in RAW-Blue™ cells determined by RT-PCR.

Figure 2: Stimulation of TLRs, NODs and Dectin-1 in RAW-Blue Cells.
A. RAW-Blue Cells were incubated with TLR and NOD agonists: TLR2 (HKLM, 1.10⁸ cells/ml), TLR1/2 (Pam3CSK4, 100 ng/ml), TLR2/6 (FSL-1, 100 ng/ml), TLR3 (poly(I:C), 10 µg/ml), TLR4 (LPS-EK, 1 µg/ml), TLR5 (RecFLA-ST, 1 µg/ml), TLR7 (CL075, 300 ng/ml), TLR9 (ODN1826, 10 µg/ml), NOD1 (Tri-DAP, 10 µg/ml), NOD2 (MDP, 10 µg/ml). After 24h incubation, TLR and NOD stimulation was assessed by measuring the levels of SEAP using QUANTI-Blue™. B. RAW-Blue Cells were stimulated with zymosan depleted of TLR activities (100 µg/ml) or heat-killed Saccharomyces cerevisiae (HKSC, 1.10⁶ cells/ml) in the presence or absence of 10 µg/ml of Anti-mDectin-1-IgG, a neutralizing monoclonal antibody against murine Dectin-1. After 24h incubation, NF-κB activation upon Dectin-1 stimulation was determined using QUANTI-Blue™.
SAFETY CONSIDERATIONS
Biosafety Level 2

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™* and *Zeocin™*

Required Selection Antibiotic(s)
- *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 strict aseptic conditions.
3. Transfer cells in a larger vial containing 15 ml of pre-warmed growth medium.
4. Centrifuge vial 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. 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% CO₂.

Frozen Stock Preparation
1. Resuspend cells at a density of 3-5 x 10⁶ cells/ml in freezing medium prepared extemporaneously with cold DMEM.
   **Note**: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.
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. After cells have recovered, subculture the cells in growth medium with an initial seeding density of 1.5 x 10⁶ cells per cm² (e.g. ~1 x 10⁶ cells in a T-75 culture flask). To maintain selection pressure, add 200 μg/ml of *Zeocin™* to the growth medium every other passage.
2. Renew growth medium twice a week.
3. Using a cell scraper, cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.
   **Note**: Do not use trypsin.

Cell handling procedure
To ensure the best results, use RAW-Blue™ cells with less than 20 passages.

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QUANTI-BLUE™ ASSAY

The QUANTI-Blue™ assay allows to detect NF-κB/AP-1 activation following activation of TLRs (with the exception of TLR5), NOD1/2 and Dectin-1.

Cell Preparation
Pass cells either 3 or 4 days prior to the reporter assay.
- If three days, seed cells at a cell density of 2.5 x 10⁶ per cm² corresponding to ~2 x 10⁶ cells in a T-75 culture flask.
- If four days, seed cells at a cell density of 1.5 x 10⁶ per cm² corresponding to ~1 x 10⁶ cells in a T-75 culture flask.

Detection of NF-κB/AP-1 activation upon PAMP stimulation

**Day 1:**
1. Remove medium and rinse twice with PBS
2. Use a cell scraper to detach cells and resuspend RAW-Blue™ cells in test medium which contains 10% (v/v) heat-inactivated FBS and prepare a cell suspension at ~550,000 cells/ml. **Note**: Some fetal bovine serum (FBS) may contain alkaline phosphatases that can interfere with SEAP quantification. To ensure that these thermosensitive enzymes are inactive, use heat-inactivated FBS (30 min at 56°C). Heat-inactivated FBS is also commercially available.
3. Add 20 μl of each agonist at various concentrations per well of a flat-bottom 96-well plate include a negative control, such as endotoxin-free water.
4. Add 180 μl of cell suspension (~100,000 cells) per well.
   **IMPORTANT**: To ensure reliable and reproducible results, homogenize the cell suspension frequently.
5. Incubate the plate at 37°C in a 5% CO₂ incubator for 18-24 h.

**Day 2:**
1. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed product data sheet.
2. Add 20 μl of induced RAW-Blue™ cells supernatant.
3. Add 180 μl of resuspended 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.

RESTRICTIONS

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.

RELATED PRODUCTS

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TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com
QUANTI-Blue™ Solution
Medium for detection and quantification of alkaline phosphatase in standard and HTS assays
Catalog code: rep-qbs, rep-qbs2
https://www.invivogen.com/quanti-blue

For research use only
Version 19F11-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. Protect QUANTI-Blue™ 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

Prepare QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml of QB buffer to 98 ml of sterile H₂O. Measure OD using a microplate reader 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. 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.

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 5 minutes. Ensure heating at 37°C does not exceed 5 minutes.

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) to wells.
6. Incubate at 37°C for 15 min to 6 h.
7. Measure optical density (OD) at 620-655 nm using a microplate reader.

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

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<th>96-well plate</th>
<th>24-well plate</th>
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<tr>
<td>QUANTI-Blue™</td>
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<tr>
<td>Supernatant</td>
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TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com

InvivoGen www.invivogen.com
**B. High Throughput Screening (HTS) procedure**

![Diagram of 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 17 ml of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml of QB buffer to 15 ml of sterile H₂O in a 50 ml screw cap tube.
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 ≤5 µl 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 to heat FBS at 56 °C for 30 min to inactivate the alkaline phosphatase activity.

**RELATED PRODUCTS**

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For a complete list of InvivoGen’s Reporter Cell Lines visit [https://www.invivogen.com/reporter-cells](https://www.invivogen.com/reporter-cells)