RAW-Blue™ ISG Cells

Mouse Macrophage Reporter Cells

Catalog code: raw-isg

https://www.invivogen.com/raw-blue-isg

For research use only

Version 19K14-MM

PRODUCT INFORMATION

Contents and Storage

- **1 vial of RAW-Blue™ ISG cells** (3-5 x 10^6 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

- Activity of RAW-Blue™ ISG cells was tested with murine type I interferon and pathogen-associated molecular patterns (PAMPs) that trigger the IFN signaling pathway, such as transfected double-stranded DNA.
- RAW-Blue™ ISG cells are guaranteed mycoplasma-free.

RESTRICTIONS

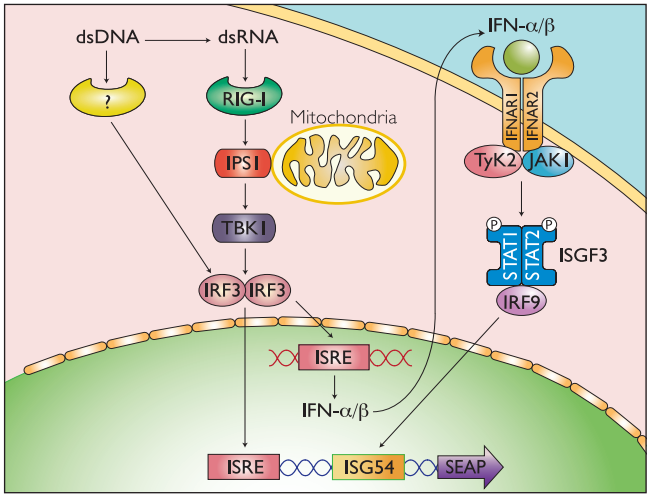
This product is covered by a Limited Use License.

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.

BACKGROUND

Interferon-alpha (IFN-α) and interferon beta (IFN-β), play an important role in viral infections. They bind to an IFN receptor complex consisting of two alpha chains (IFNAR1 and IFNAR2) and recruit JAK1 and TyK2. These kinases phosphorylate STAT1 and STAT2 leading to the formation of the ISGF3 complex. ISGF3 binds to IFN-stimulated response elements (ISRE) in the promoters of IFN-stimulated genes (ISG) to regulate their expression. IFN-α and IFN-β are produced in response in particular to viral pathogen associated molecular patterns (PAMPs), such as viral RNA and DNA. These PAMPS, such as transfected poly (dA:dT) and 5'ppp-dsRNA, are recognized by cytoplasmic pattern recognition receptors including the RNA helicase RIG-I. Stimulation of RAW-Blue™ ISG cells with these compounds triggers the secretion of IFN-α and IFN-β.



PRODUCT DESCRIPTION

RAW-Blue™ ISG cells allow the detection of bioactive murine type I IFNs or PAMPs by monitoring the activation of the interferon regulatory factor (IRF) pathway. They are derived from the RAW 264.7 macrophages. RAW-Blue™ ISG cells stably express a secreted embryonic alkaline phosphatase (SEAP) gene under the control of the interferon-stimulated gene 54 (ISG54)-inducible promoter enhanced by a multimeric IFN-stimulated response elements (ISRE). Stimulation of these cells with mIFN- or mIFN-β, or type I IFN inducers, such as tranfected poly(dA:dT) or 5'ppp-dsRNA, can activate the IRF3, 7 or 9 pathways and trigger the subsequent production of SEAP. Levels of SEAP in the supernatant can be easily determined with QUANTI-Blue™, a SEAP detection medium. RAW-Blue™ ISG cells are resistant to Zeocin™.

- Detection range for mIFN-α: $5x10^2$ - $5x10^4$ IU/ml
- Detection range for mIFN-β: 10^1 - 10^3 IU/ml

TECHNICAL SUPPORT

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

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FAQ Any questions about our cell lines?

Visit our FAQ page.

InvivoGen

www.invivogen.com

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 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 3-5 x 10^6 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 10^4 cells per cm^2 (e.g. ~1 x 10^6 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™ ISG cells with less than 20 passages.

DETECTION OF IRF ACTIVATION

RAW-Blue™ ISG cells can detect any compound that activates the IRF3/7/9 pathway.

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 10^4 per cm^2 corresponding to ~ 2 x 10^6 cells in a T-75 culture flask.
- If four days, seed cells at a cell density of 1.5 x 10^4 per cm^2 corresponding to ~ 1 x 10^6 cells in a T-75 culture flask.

Day 1:

1. Sample preparation: Prepare a working dilution range of your samples in endotoxin-free water.
2. Cell suspension preparation:
- Remove medium from RAW-Blue™ ISG cells and rinse twice with warm PBS.
- Use a cell scraper to detach cells and resuspend cells in test medium which contains 10% (v/v) heat-inactivated FBS and prepare a cell suspension at ~550,000 cells/ml.
3. Add 20 µl of your sample per well of a flat-bottom 96-well plate.
4. Add 20 µl of a positive control (such as murine Type I IFN) in another well.
5. Add 20 µl of negative control (or Test Medium) in another well.
6. Add 180 µl of cell suspension (~100,000 cells) per well.
IMPORTANT: To ensure reliable and reproducible results, homogenize the cell suspension.
7. Incubate the plate at 37°C in a 5% CO_2 incubator for 18-24 h.

Day 2:

1. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed product data sheet.
2.Add 180 µl of resuspended QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
3. Add 20 µl of induced RAW-Blue™ ISG cells supernatant.
4. Incubate the plate at 37°C for 1-6 h.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Catalog Code
5'ppp-dsRNA	tlrl-3prna
5'ppp-dsRNA Control	tlrl-3prnac
LyoVec™ (transfection reagent)	lyec-12
Normocin™	ant-nr-1
Poly(dA:dT)/LyoVec™	tlrl-patc
Poly(I:C)	tlrl-pic
QUANTI-Blue™	rep-qb1
Zeocin™	ant-zn-1

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

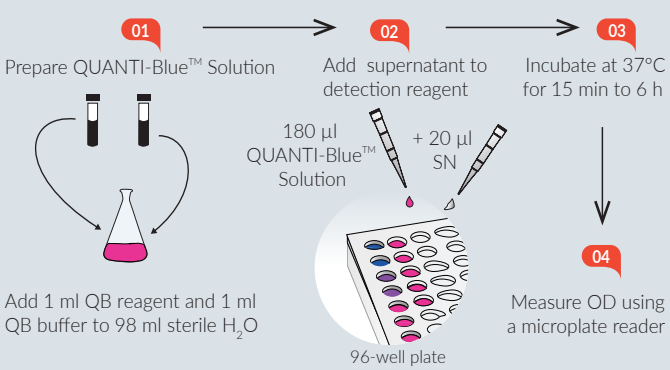

Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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 (HTS) procedure

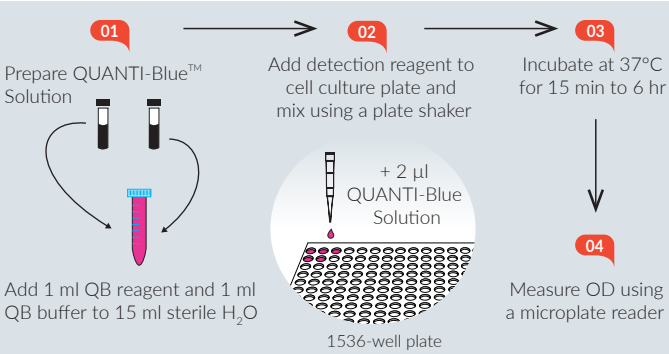

Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

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 water 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

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
https://www.invivogen.com/reporter-cells

TECHNICAL SUPPORT
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