B16-Blue™ ISG-KO-STING Cells

STING Knockout IRF-Inducible SEAP Reporter B16 Melanocytes

Catalog code: bb-kostg

https://www.invivogen.com/b16-blue-isg-ko-sting

For research use only

Version 19E16-MM

PRODUCT INFORMATION

Contents

• **1 vial of B16-Blue™ ISG-KO-STING 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 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). Store QB reagent and QB buffer 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.

B16-Blue™ ISG-KO-STING Cells should not be passaged more than 20 times to remain fully efficient. B16-Blue™ ISG-KO-STING Cells should be maintained in growth medium supplemented with the selective antibiotic, Zeocin™ (100 µg/ml). Antibiotic pressure with Zeocin™ is required to maintain the plasmid coding for SEAP.

Quality control

• STING knockout is verified by functional assays and DNA sequencing.

• The stability for 20 passages, following thawing, has been verified.

• These cells are guaranteed mycoplasma-free.

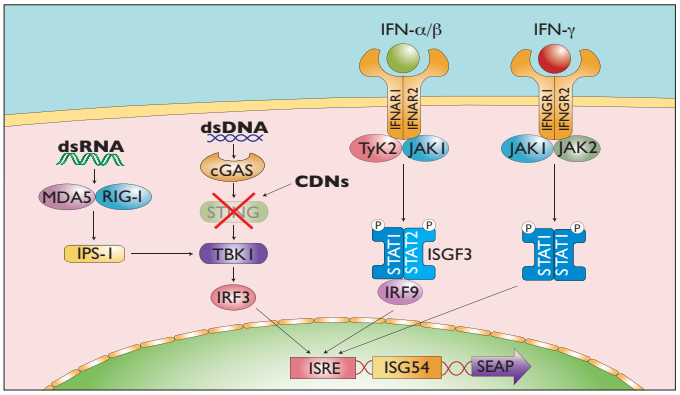
USE RESTRICTIONS

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 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

The presence of nucleotides (dsDNA, dsRNA, or cyclic dinucleotides (CDNs)) in the cytoplasm of mammalian cells triggers immune responses such as the production of interferons (IFNs). Cytosolic DNA is detected by cytosolic DNA sensors, including cGAS, leading to the induction of type I IFNs through the STING-TBK1-IRF3 pathway[1]. Viral dsRNA is detected by cytoplasmic RNA helicases, RIG-I and MDA-5, that interact with the IPS-1 adaptor protein and trigger IFN production through TBK1-IRF3 signaling. CDNs bind directly to STING leading to IRF3-mediated IFN production[2]. IFN-α and IFN-β are type I IFNs that play an important role in viral infections, while IFN-γ is a type II IFN that plays a role in anti-microbial responses. Upon binding to their respective receptors, IFNs activate the JAK-STAT pathway with the subsequent activation of IFN-stimulated response elements (ISRE) in the promoters of IFN-stimulated genes (ISG).



CELL LINE DESCRIPTION

B16-Blue™ ISG-KO-STING cells were generated from B16-Blue™ ISG cells, murine B16-F1 melanoma-derived cells, through the stable knockout of the STING gene. They express a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of the I-ISG54 promoter which is comprised of the IFN-inducible ISG54 promoter enhanced by a multimeric ISRE.

B16-Blue™ ISG-KO-STING and B16-Blue™ ISG cells can be used to study STING signaling and for the detection of bioactive murine types I and II IFNs by monitoring the activation of the JAK/STAT pathway. Unlike the parental cells, B16-Blue™ ISG-KO-STING cells do not respond to cytosolic DNA, DMXAA, canonical and non-canonical CDNs while retaining the ability to respond to type I and type II IFNs. Stimulation of these cells with IFN triggers the activation of the I-ISG54 promoter and the production of SEAP. Levels of SEAP in the supernatant can be easily determined using QUANTI-Blue™, a SEAP detection reagent.

B16-Blue™ ISG-KO-STING cells are resistant to Zeocin™.

1. Sun L., 2013. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science 339(6121):786-91. **2. Burdette DL. 2011.** STING is a direct innate immune sensor of cyclic di-GMP. Nature 478(7370):515-8.

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FAQ Any questions about our cell lines ?
Visit our FAQ page

SAFETY CONSIDERATIONS

Biosafety Level 1

HANDLING PROCEDURES

Required Cell Culture Medium

• **Growth Medium:** DMEM, 10% (v/v) heat-inactivated FBS (30 min at 56°C), 100 U/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml Normocin™, 2 mM L-glutamine

Note: Heat-inactivated FBS is also commercially available.

• **Freezing Medium:** DMEM, 20% FBS, 10% (v/v) DMSO

Required Selective 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.

- 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 (approximately 2 minutes).
- 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 of the operations from this point should be carried out under strict aseptic conditions.

- Transfer cells in a larger vial containing 15 ml of pre-warmed growth medium. **Do not add selective antibiotics until the cells have been passaged twice.**
- Centrifuge vial at 1000-1200 RPM (RCF = 200-300 g) for 5 minutes.
- Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selective antibiotics.
- Transfer the vial contents to a T-25 tissue culture flask containing 5 ml of growth medium without selective antibiotics.
- Place the culture at 37 °C in 5% CO2.

Frozen Stock Preparation

- Resuspend cells at a density of 3-5 x 10^6 cells/ml in freezing medium prepared extemporaneously with cold growth medium.

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

- Aliquot 1 ml cells into cryogenic vials.
- Place vials in a freezing container and store at -80 °C overnight.
- Transfer vials to liquid nitrogen for long term storage.

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

Cell maintenance

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

Notes:

- B16-Blue™ ISG-KO-STING cells produce melanin causing the culture medium to appear dark brown or black, especially when approaching a high level of confluence.

- Use B16-Blue™ ISG-KO-STING cells with less than 20 passages.

Reporter Assay

Day 1:

- Add 20 µl of each sample per well of a flat-bottom 96-well plate.
- Add 20 µl of positive control, such as mIFN, in one well.
- Add 20 µl of negative control (growth medium or sterile PBS) in one well.
- Prepare a cell suspension of B16-Blue™ ISG-KO-STING cells at ~420,000 cells per ml in growth medium (containing 10% v/v heat-inactivated FBS).
- Add 180 µl of cell suspension (~75,000 cells) per well.
- Incubate the plate at 37 °C in a 5% CO_2 incubator for 20-24 h.

Day 2:

- Prepare QUANTI-Blue™ Solution following the instructions on the enclosed product data sheet.
- Add 180 µl of QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
- Add 20 µl of induced B16-Blue™ ISG-KO-STING cells supernatant.
- Incubate the plate at 37 °C incubator for 1-5 h.
- Determine SEAP levels using a spectrophotometer at 620-655 nm.

Murine IFN Detection range

• **mIFN-α:** 10^2 - 10^4 IU/ml
• **mIFN-β:** 10^2 - 10^4 IU/ml
• **mIFN-γ:** 0.1 ng - 1 µg/ml

RELATED PRODUCTS

Product	Catalog Code
2'3'-cGAMP	tlrl-nacga23
3'3'-cGAMP	tlrl-nacga
B16-Blue™ ISG (Parental cells)	bb-ifnabg
DMXAA	tlrl-dmx
Normocin™	ant-nr-1
QUANTI-Blue™ Solution	rep-qbs
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 18L10-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

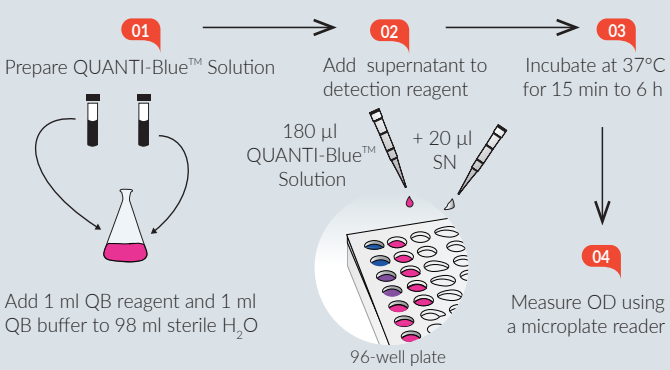

Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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

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B. High Throughput Screening procedure

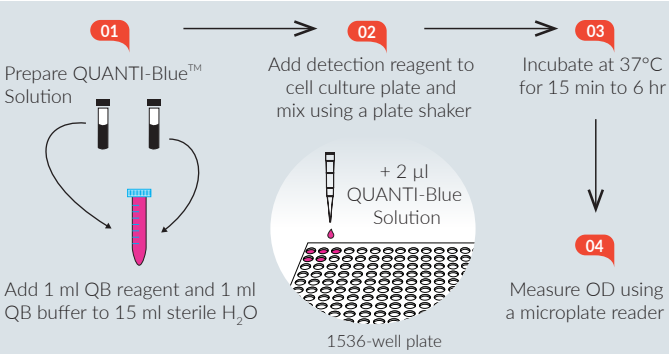

Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

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®)	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

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