HEK-Blue™ ISG KO-STING Cells

STING-knockout interferon regulatory factor (IRF)-inducible SEAP reporter HEK293 cells

Catalog code: hkb-isgkostg

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

For research use only

Version 18I06-MM

PRODUCT INFORMATION

Contents:

• **1 vial of HEK-Blue™ ISG KO-STING cells** (3-7 x 10^6 cells)

IMPORTANT: Cells are shipped frozen. If cells 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 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). 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.

Handling Cells Upon Receipt

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.

HEK-Blue™ ISG KO-STING cells should not be passaged more than 20 times to remain fully efficient. HEK-Blue™ ISG-KO-STING cells should be maintained in growth medium supplemented with Zeocin™. Antibiotic pressure with Zeocin™ is required to maintain the plasmid coding for SEAP.

QUALITY CONTROL

• STING knockout has been verified by functional assays (see validation sheet) and DNA sequencing.

• The stability of this cell line for 20 passages, following thawing, has been verified.

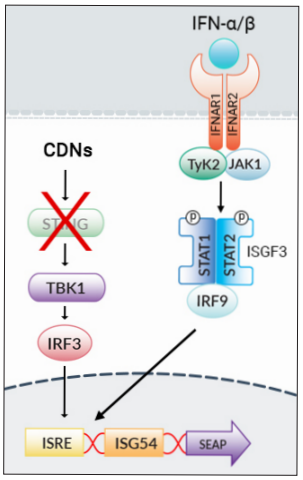
• These cells are guaranteed mycoplasma-free.

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.

BACKGROUND



STING (stimulator of interferon genes; also known as TMEM173, MITA, MPYS and ERIS) is essential for the interferon (IFN) response to cytosolic nucleic acids, such as microbial or self-DNA[1], and acts as a direct sensor of cyclic dinucleotides (CDNs)[2].

CDNs are important second messenger molecules in bacteria, affecting numerous responses of the prokaryotic cell. In mammalian cells, CDNs act as agonists of the innate immune response[3]. CDNs bind directly to and activate STING leading to TANK Binding Kinase 1 (TBK1)-dependent interferon regulatory factor 3 (IRF3) activation and type I IFN production. IFNs then initiate the JAK-STAT pathway, with subsequent activation of IFN-stimulated response elements (ISRE) in the promoters of IFN-stimulated genes (ISG).

1. Ishikawa H. & Barber G., 2008. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature 455, 674–678. **2. Burdette DL. *et al.*, 2011.** STING is a direct innate immune sensor of cyclic di-GMP. Nature 478(7370):515-8. **3. Wu J. *et al.*, 2013.** Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. Science 339(6121):826-30.

CELL LINE DESCRIPTION

HEK-Blue™ ISG KO-STING cells were specifically designed to study the STING/TBK1/IRF3 signaling pathway. HEK-Blue™ ISG KO-STING cells were generated from HEK-Blue™ ISG cells through stable knockout of the STING gene. The parental HEK-Blue™ ISG cells were derived from the PEAKrapid cell line (similar to ATCC® CRL-2828™) which itself was derived from the HEK293 cell line. Unlike their parental cell line, HEK-Blue™ ISG KO-STING cells do not respond to cytosolic DNA and CDNs.

HEK-Blue™ ISG KO-STING cells can detect bioactive human type I IFNs through the activation of the JAK-STAT-IRF9 pathway. HEK-Blue™ ISG KO-STING cells express a secreted embryonic alkaline phosphatase (SEAP) under the control of the IRF-inducible promoter comprised of five ISREs fused to an ISG54 minimal promoter. Levels of SEAP in the supernatant can be easily determined with QUANTI-Blue™, a SEAP detection reagent.

HEK-Blue™ ISG KO-STING cells are resistant to Zeocin™ and hygromycin B. Cells should be maintained in growth medium (see next page) supplemented with Zeocin™. Antibiotic pressure with Zeocin™ is required to maintain the plasmid coding for SEAP.

TECHNICAL SUPPORT
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SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ ISG KO-STING cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) that require Biosafety Level 2 according to CDC guidelines. The biosafety level may vary depending on the country.

HANDLING PROCEDURES

Required Cell Culture Medium

• Growth Medium: DMEM, 4.5 g/l glucose, 2 mM L-glutamine 10% (v/v) fetal bovine serum (FBS), Pen-Strep (100 U/ml-100 µg/ml), 100 µg/ml Normocin™

• Freezing Medium: DMEM with 20% FBS and 10% (v/v) DMSO

• Test Medium: DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% (v/v) heat-inactivated FBS (30 min at 56 °C), Pen-Strep (100 U/ml-100 µg/ml), 100 µg/ml Normocin™

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.

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

3. Transfer cells to a tube containing 15 ml of pre-warmed growth medium. **Do not add Zeocin™ until the cells have been passaged twice.**
4. Centrifuge tube 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 without selective antibiotics.
6. Transfer the contents to a T-25 tissue culture flask containing 5 ml of growth medium without selective antibiotics.
7. Place the culture at 37 °C in 5% CO_2.

Note: Following thawing, adherence to the plastic surface and proliferation may be slow. In the initial culture procedure, these cells usually take 3-4 days before reaching confluency in a T-25 tissue culture flask.

Frozen Stock Preparation

1. Resuspend cells at a density of 3-5 x 10^6 cells/ml in freshly prepared freezing medium.

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

2. Dispense 1 ml of cell suspension 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 and subculture the cells in growth medium supplemented with 100 µg/ml of Zeocin™.
2. Renew growth medium twice a week.
3. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

Note: A subcultivation ratio of 1:2 to 1:8 is recommended. As a general guide

1:2 split should be 70-80% confluent in 1 day
1:4 split should be 70-80% confluent in 2 days
1:8 split should be 70-80% confluent in 4 days

Reporter Assay

Day 1:

Use HEK-Blue™ ISG KO-STING cells with their corresponding parental (wild-type) cell line, HEK-Blue™ ISG cells.

1. Add 20 µl of each sample per well of a flat-bottom 96-well plate.
2. Add 20 µl of a positive control such as human IFN-β at 10^3 U/ml (final concentration) in one well.
3. Add 20 µl of a STING ligand such as 2'3'-cGAMP at 10 mg/ml (final concentration) in one well.

Note: This ligand will induce SEAP activity in the parental HEK-Blue™ ISG cells but not in HEK-Blue™ ISG KO-STING cells.

4. Add 20 µl of a negative control such as LPS-EK Ultrapure (lipopolysaccharide from *E. coli* K12 strain; a TLR4 ligand) at 100 ng/ml (final concentration) or growth medium in one well.
5. Prepare a cell suspension of HEK-Blue™ ISG KO-STING cells at ~280,000 cells per ml in test medium (containing 10% v/v heat-inactivated FBS).

Note: Some FBS may contain alkaline phosphatases that can interfere with SEAP quantification. We recommend to use heat-inactivated FBS to inactivate these enzymes which are thermosensitive.

6. Add 180 µl of cell suspension (~50,000 cells) per well.
7. Incubate the plate at 37 °C in a CO_2 incubator for 20-24 h.

Day 2:

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

RELATED PRODUCTS

Product	Catalog Code
2'3'-cGAMP	tlrl-nacga23
2'3'-c-di-AMP	tlrl-nacda23
HEK-Blue™ ISG cells	hkb-isg-1
LPS-EK Ultrapure	tlrl-peklps
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

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

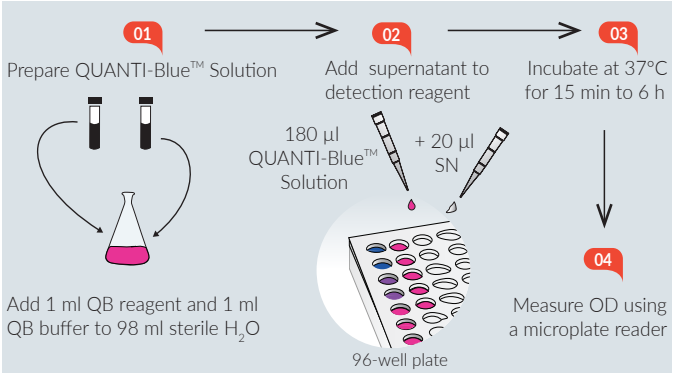

Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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

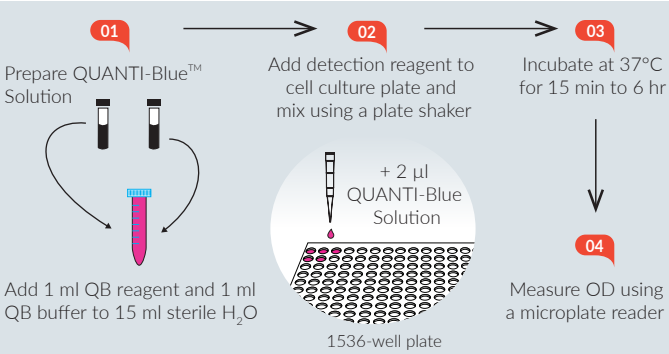

Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

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