HEK-Blue™ STAT6-hSTING-R232 Cells

Human STING (variant R232)-STAT6 reporter cells

Catalog code: hkb-st6r232

https://www.invivogen.com/sting-r232-stat6-cells

For research use only

Version 23K27-MM

PRODUCT INFORMATION

Contents:

- **3-7 x 10^6 HEK-Blue™ STAT6-hSTING-R232 cells in a cryovial or shipping flask.** IMPORTANT: If cells provided in a cryovial are not frozen upon arrival, contact InvivoGen immediately.
- **2 x 1 ml HEK-Blue™ Selection** (250X concentrate). A solution containing the required selection antibiotics. Store at 4°C or -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.

Handling Frozen 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.

IMPORTANT: For cells that arrive in a shipping flask please refer to the enclosed 'cell recovery procedure'.

Cell Line Stability

Cells will undergo genotypic changes over time that will result in reduced responsiveness 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.

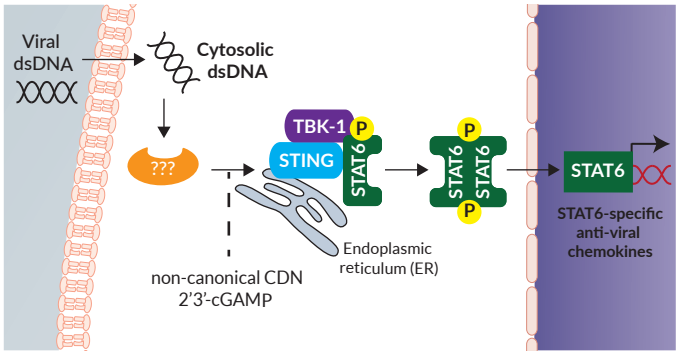
Quality Control

- STAT6-dependent SEAP reporter activity in response to various STING ligands and other cytokines has been validated.
- The stability of this cell line for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

BACKGROUND

STING (stimulator of interferon genes) is essential for an effective response to cytoplasmic foreign or self-DNA. It directly senses cyclic dinucleotides (CDNs), which are important messengers in bacteria and innate immune agonists in mammals[1]. The most prevalent variant of human STING, referred to as 'wild-type', is the R232 allele[2]. The activation of STING causes a TANK-binding-kinase-I (TBK1)-dependent cascade, ultimately, leading to IFN regulatory factor (IRF3)-dependent type I IFN production and NF-κB-dependent inflammatory cytokine production[1].

Additionally, signal transducer and activator of transcription 6 (STAT6) has been reported to be recruited to STING for TBK1-mediated phosphorylation during viral infection[3]. Ultimately, the activation of STAT6 induces a specific set of anti-viral chemokines responsible for immune cell homing, which is essential to reduce viral replication[3]. Notably, STING-dependent activation of STAT6 was found to be janus kinase (JAK)-independent and is thus, distinct from the 'canonical' STAT6 pathway activated by IL-4 and IL-13, which is crucial in adaptive immunity[3].



1. Cheng Z. *et al.* 2020. The interactions between cGAS-STING pathway and pathogens. Signal Transduct Target Ther 5, 91. 2. Yi G. *et al.* 2013. Single nucleotide polymorphisms of human STING can affect innate immune response to cyclic dinucleotides. PLoS One 8, e77846. 3. Chen H. *et al.* 2011. Activation of STAT6 by STING is critical for antiviral innate immunity. Cell 147, 436-446.

CELL LINE DESCRIPTION

HEK-Blue™ STAT6-hSTING-R232 cells are specifically designed to monitor the activation of the STAT6 signaling pathway induced by STING. These cells were generated by stable transfection and overexpression of the wild-type human (h)STING variant R232 in a human embryonic kidney (HEK)293-derived cell line that expresses human STAT6, and a STAT6-inducible secreted embryonic alkaline phosphatase (SEAP) reporter. The other genes of this signaling pathway are naturally expressed at sufficient levels. Numerous studies have indicated that type I interferons (IFNs) can activate STAT6, thus, HEK-Blue™ STAT6-hSTING-R232 cells have IFN-α-R2 knocked out and subsequently, do not respond to type I IFNs, such as IFN-α and IFN-β. HEK-Blue™ STAT6-hSTING-R232 cells stably express SEAP under the control of the IFN-β minimal promoter fused to four STAT6 binding sites. Thus, activation of the STAT6 pathway in these cells by hSTING agonists (i.e. 2'3'cGAMP) induces the expression of SEAP, which is easily detectable in the cell culture supernatant with QUANTI-Blue™ Solution, a SEAP detection reagent. HEK-Blue™ STAT6-hSTING-R232 cells are resistant to Blasticidin, Hygromycin B, and Zeocin®.

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

SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Blue™ STAT6-hSTING-R232 cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) that require **Biosafety level 2** according to the American Center for Disease Control and Prevention (CDC) guidelines. The biosafety level may vary depending on the country. For example, in Germany HEK293 cell lines are designated Biosafety Level 1 according to the Central Committee of Biological Safety, Zentrale Kommission für die Biologische Sicherheit (ZKBS). Please check with your country's regulatory authority regarding the use of these cells.

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% (v/v) heat-inactivated fetal bovine serum (FBS; 30 min at 56°), 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, Pen-Strep (100 U/ml-100 µg/ml) **without HEK-Blue™ Selection and Normocin™**

Required Selective Antibiotics

- HEK-Blue™ Selection

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% (v/v) 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. **Do not add selective antibiotics until the cells have been passaged twice.**
4. Centrifuge vial at 300 x g (RCF) 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 vial contents to a 25 cm^2 tissue culture flask containing 5 ml of growth medium without selective antibiotics.
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 freshly prepared with cold growth medium.

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

2. Prepare 1 ml aliquots of cells in 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

- Maintain and subculture the cells in growth medium supplemented with HEK-Blue™ Selection.
- 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.

REPORTER ASSAY

Day 1:

1. Add 20 µl of each sample per well of a flat-bottom 96-well plate.
2. Add 20 µl of recombinant human IL-4 (1 ng/ml) or recombinant human IL-13 (10 ng/ml) as positive controls in one well.
3. Add 20 µl of TNF-α at 100 ng/ml (negative control, other cytokines can be used) in one well.
4. Prepare a cell suspension of HEK-Blue™ STAT6-hSTING-R232 cells (~3 x 10^5 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.

5. Add 180 µl of cell suspension (~50,000 cells) per well.
6. 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 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 HEK-Blue™ STAT6-hSTING-R232 cell supernatant.
4. Incubate the plate at 37 °C for 1-3 h.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

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 with 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

Product	Catalog Code
2'3'-cGAMP	tlrl-nacga23
2'3'-cGAM(PS)2 (Rp/Sp)	tlrl-nacga2srs
3'3'-cGAMP	tlrl-nacga
cAIM(PS)2 Difluor (Rp/Sp)	tlrl-nacairs
HEK-Blue™ Selection	hb-sel
Normocin™	ant-nr-1
QUANTI-Blue™ Solution	rep-qbs
Recombinant human IL-4	rcyec-hil4
THP1-Dual™ KI-hSTING-R232	thpd-r232
THP1-Dual™ KO-STING	thpd-kostg

QUANTI-Blue™ Solution

Medium for detection and quantification of alkaline phosphatase in standard and HTS assays

Catalog code: rep-qbs, rep-qbs2, rep-qbs3

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

For research use only

Version 23C09-MM

PRODUCT INFORMATION

Contents: QUANTI-Blue™ Solution is available in three pack sizes

- **rep-qbs:** 5 x 1 ml of QB reagent and 5 x 1 ml QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **25 x 96-well plates** (500 ml using the standard procedure) or **20 x 1536-well plates** (85 ml using the HTS screening procedure).
- **rep-qbs2:** 10 x 1 ml of QB reagent and 10 x 1 ml QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **50 x 96-well plates** (1 L using the standard procedure) or **40 x 1536-well plates** (170 ml using the HTS screening procedure).
- **rep-qbs3:** 1 x 20 ml bottle of QB reagent and 1 x 20 ml bottle of QB buffer, sufficient to prepare QUANTI-Blue™ Solution for **100 x 96-well plates** (2 L using the standard procedure) or **80 x 1536-well plates** (340 ml using the HTS screening procedure).

Required Material (not provided)

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

Storage and stability

- Product is shipped at room temperature. Upon receipt, store QB reagent and QB buffer at -20 °C. Product is stable for 1 year at -20 °C when properly stored.
- The 20 ml bottles of QB reagent and QB buffer are designed for single use. If required, individual aliquots of QB reagent and QB buffer can be prepared upon receipt or following a single freeze-thaw cycle. Store aliquots at -20°C. **Avoid repeated freeze-thaw cycles**.

Note: During storage, a precipitate may form in the 20 ml bottle of QB reagent and QB buffer. If this occurs, heat the product at 37°C for 30 seconds and vortex until the precipitate disappears. The formation of a precipitate does not affect the activity of the product.

- Reconstituted QUANTI-Blue™ Solution is stable for 2 weeks at 2-8 °C and for 2 months at -20°C. Protect from light.

Quality Control

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

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

QUANTI-Blue™ is highly sensitive for quantitative measurement. It has a higher saturation threshold than with pNPP (p-nitrophenyl phosphate) resulting in more significant differences between no, low or high AP activity. Another advantage of QUANTI-Blue™ is that it can determine secreted AP activity without disturbing cells, thus allowing the repeated sampling of cell cultures for kinetic studies.

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

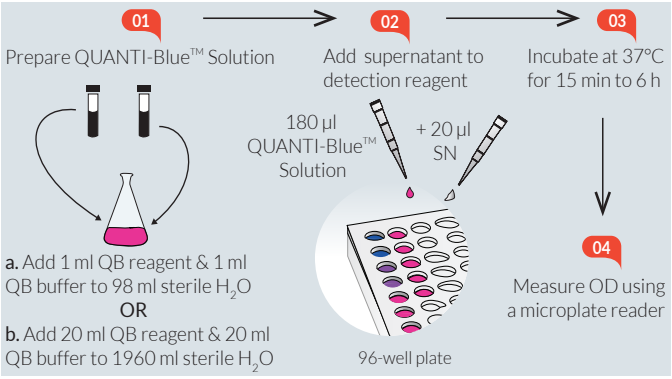

Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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. In a sterile bottle or flask, prepare QUANTI-Blue™ Solution by adding:
   a. **1 ml** of QB reagent and **1 ml** of QB buffer to **98 ml** of sterile water.
   b. **20 ml** of QB reagent and **20 ml** of QB buffer to **1960 ml** of sterile water.
2. Mix 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 the 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 heating 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

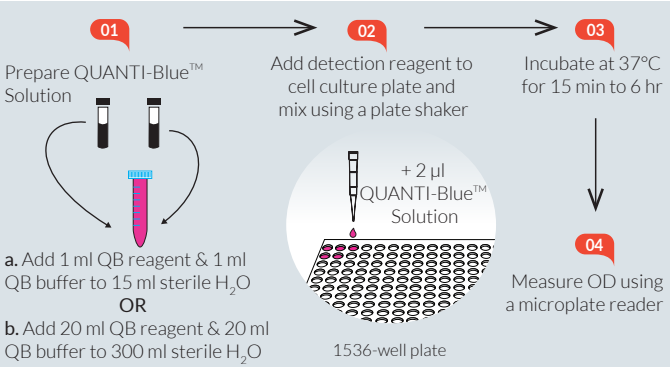

Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

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 QUANTI-Blue™ Solution by adding:
   a. **1 ml** of QB reagent and **1 ml** of QB buffer to **15 ml** of sterile water in a sterile 50 ml screw cap tube.
   b. **20 ml** of QB reagent and **20 ml** of QB buffer to **300 ml** of sterile water in a sterile glass bottle or flask.
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 heating 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
https://www.invivogen.com/reporter-cells