THP1-Dual™ KO-STING Cells
STING knockout NF-κB-SEAP and IRF-Lucia luciferase reporter monocytes
Catalog code: thpd-kostg
http://www.invivogen.com/thp1-dual-kosting
For research use only
Version 18D16-MM

INTRODUCTION
STING (stimulator of interferon genes), alternatively known as MPYS, TMEM173, MITA and ERIS, is a key sensor of cytosolic nucleic acids. STING, initially thought to serve solely as an adaptor protein for mediating signaling by cytosolic DNA sensors (CDS), was found to be a direct sensor of cyclic dinucleotides (CDNs). CDNs are ubiquitous second messenger molecules used in bacterial signal transduction and are defense triggers in mammalian cells. Upon bacterial pathogen attack, CDNs released into cells bind directly to STING leading to TBK1-mediated IRF3 activation and type I interferon (IFN) production. Type I IFNs activate the JAK-STAT pathway with the subsequent activation of IFN-stimulated response elements (ISRE) in the promoters of IFN-stimulated genes (ISG).

PRODUCT INFORMATION
Contents and Storage
• 1 vial of THP1-Dual™ KO-STING cells (3-7 x 10⁶ cells) in freezing medium

IMPORTANT: Cells are shipped frozen. If cells are not frozen upon arrival, contact InvivoGen immediately.

• 1 ml of Blasticidin (10 mg/ml). Store at 4°C or at -20°C.*
• 1 ml of Zeocin™ (100 mg/ml). Store at 4°C or at -20°C.*
• 1 ml of Normocin™ (50 mg/ml). Normocin™ is a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20°C.*

*The expiry date is specified on the product label.

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.

THP1-Dual™ KO-STING cells were generated from THP1-Dual™ cells by stable knockout of the STING gene. They derive from human THP-1 monocytes, a cell line often used to study DNA sensing pathways as they express all the cytosolic DNA sensors identified so far (with the exception of DAI). THP1-Dual™ and THP1-Dual™ KO-STING cells stably express two inducible secreted reporter genes: Lucia luciferase and SEAP (secreted embryonic alkaline phosphatase). The Lucia luciferase reporter gene is under the control of an ISG54 (interferon-stimulated gene) minimal promoter in conjunction with five ISG54 (interferon-stimulated gene) minimal promoter. Both reporter proteins are readily measurable in the cell culture supernatant when using QUANTI-Blue™ Solution reagent and QUANTI-Blue™ Solution reagent.

THP1-Dual™ KO-STING cells are resistant to blasticidin and Zeocin™. RIG-I ligands such as transfected Poly(I:C).

THP1-Dual™ KO-STING and THP1-Dual™ cells can be used to study the role of STING by monitoring IRF-induced Lucia luciferase activity. Unlike the parental cells, THP1-Dual™ KO-STING cells exhibit no detectable response to cytosolic DNA and CDNs while retaining the ability to respond to type I IFNs. As expected these cells remain responsive to RIG-I ligands such as transfected Poly(I:C). THP1-Dual™ KO-STING cells are resistant to blasticidin and Zeocin™.
SAFETY CONSIDERATIONS
Biosafety Level: 1

HANDLING PROCEDURES

Required Cell Culture Medium
- Growth Medium: RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% (v/v) 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: 90% fetal bovine serum (FBS), 10% DMSO
- Test Medium: RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated fetal bovine serum, Pen-Strep (100 U/ml-100 µg/ml)

Initial culture of all THP-1 derived cells must be performed in growth medium containing 20% heat-inactivated FBS.
Note: The use of Normocin™ together with Pen-Strep is required to keep the cells free of microbial contaminants. Contamination of this cell line may activate TLRs resulting in differentiation of the monocytes and activation of the reporter gene.

Requisite Selective Antibiotics
- Blasticidin and 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 aseptic conditions.
3. Transfer cells in a vial containing 15 ml of pre-warmed growth medium (with 20% heat-inactivated FBS). Do not add selective antibiotics until the cells have been passaged twice.
4. Centrifuge vial at 1000-1500 RPM (RCF 200 - 300 g) for 5 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium (with 20% heat-inactivated FBS).
6. Transfer the vial contents to a 25 cm² tissue culture flask containing 5 ml of growth medium (with 20% heat-inactivated FBS).
7. Place the culture at 37°C, 5% CO₂.

Frozen Stock Preparation
1. Resuspend cells at a density of 5-7 x 10⁶ cells/ml in freshly prepared freezing medium.
2. Dispense 1 ml of the cell suspension into cryogenic vials.
3. Place vials in a freezing container and store at -80°C overnight.
4. Centrifuge vial at 1000-1500 RPM (RCF 200 - 300 g) for 5 minutes.
5. Remove supernatant and resuspend THP1-Dual™ KO-STING cells at 5 x 10⁶ cells/ml in fresh, pre-warmed growth medium.

DETECTION OF NF-kB ACTIVATION
1. Add 20 µl of sample per well including a positive control (such as recombinant human TNF-α at 1 ng/ml) and endotoxin free water as a negative control (use new tips for each well to avoid cross-contamination).
2. Add 180 µl of cell suspension (~100,000 cells) per well of a flat-bottom 96-well plate.
3. Incubate the plate for 18-24 h at 37°C, 5% CO₂.
4. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed data sheet.
5. Add 180 µl of resuspended QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
6. Add 20 µl of THP1-Dual™ KO-STING cells supernatant.
7. Incubate the plate at 37°C for 1-6 h.
8. Determine SEAP levels using a spectrophotometer at 620-655 nm.

DETECTION OF IFN INDUCTION
Below is a protocol for end-point readings using a luminometer with an injector. This protocol can be adapted for use with a luminometer with or without an injector for kinetic measurements.

1. Add 20 µl of sample per well including a positive control (such as recombinant human IFN-β at 1000 IU/ml) and endotoxin free water as a negative control (use new tips for each well to avoid cross-contamination).
2. Add 180 µl of cell suspension (~100,000 cells) per well of a flat-bottom 96-well plate.
3. Incubate the plate for 18-24 h at 37°C, 5% CO₂.
4. Prepare QUANTI-Luc™ following the instructions on the enclosed data sheet.
5. Set the luminometer with the following parameters: 50 µl of injection, end-point measurement with a 4 second start time and 0.1 second reading time.
6. Pipet 10 µl of THP1-Dual™ KO-STING cell culture medium per well into a 96-well white (opaque) or black plate, or a luminometer tube.
7. Prime the injector with the QUANTI-Luc™ assay solution and proceed with the measurement.

RELATED PRODUCTS

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Cat. Code</th>
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</thead>
<tbody>
<tr>
<td>Blasticidin</td>
<td>Selective antibiotic</td>
<td>ant-bl-1</td>
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<td>LyoVec™</td>
<td>Transfection reagent</td>
<td>lye-12</td>
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<tr>
<td>Normocin™</td>
<td>Antimicrobial agent</td>
<td>ant-nr-1</td>
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<td>QUANTI-Blue™ Solution</td>
<td>SEAP detection medium</td>
<td>rep-qs1</td>
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<td>QUANTI-Luc™</td>
<td>Luciferase detection medium</td>
<td>rep-q1l</td>
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<td>THP1-Dual™</td>
<td>Parental cell line</td>
<td>thpd-nfs</td>
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<td>2′,3′-cGAMP</td>
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<td>dsDNA &amp; transfection reagent</td>
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<td>dsRNA &amp; transfection reagent</td>
<td>tlr-pacv</td>
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<td>dsRNA</td>
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<td>Poly(LC)(HMW)/LyoVec™</td>
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<td>tlr-patc</td>
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<td>FIA-ST Utralpure</td>
<td>Flagellin; TLR5 ligand</td>
<td>tlr-epatla</td>
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<tr>
<td>Pam3CSK4</td>
<td>Lipoprotein; TLR1/2 ligand</td>
<td>tlr-pms</td>
</tr>
</tbody>
</table>

TECHNICAL SUPPORT
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E-mail: info@invivogen.com

www.invivogen.com
QUANTI-Luc™
A coelenterazine-based luminescence assay reagent
Catalog code: rep-qlc1, rep-qlc2
http://www.invivogen.com/quanti-luc
For research use only
Version 18D30-MM

PRODUCT INFORMATION

Contents
QUANTI-Luc™ is provided as packs of individually sealed pouches.
• rep-qlc1: 2 pouches of QUANTI-Luc™
• rep-qlc2: 5 pouches of QUANTI-Luc™
Each pouch contains everything needed to prepare 25 ml of reagent allowing the preparation of 500 wells of a 96-well plate.

Storage and Stability
- Store QUANTI-Luc™ pouches at -20°C for 12 months.
- Reconstituted QUANTI-Luc™ is stable for 1 week at 4°C and for 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.

Note: This product is photosensitive and should be protected from light.

DESCRIPTION

QUANTI-Luc™ is an assay reagent containing all the components required to quantitatively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases. QUANTI-Luc™ contains the coelenterazine substrate and stabilizing agents for the luciferase reaction. The light signal produced is quantified using a luminometer and expressed as relative light units (RLU). The signal produced correlates to the amount of luciferase protein expressed, indicating promoter activity in the reporter assay.

QUANTI-Luc™ is optimized for use with Lucia luciferase reporter cell lines. Lucia luciferase is a secreted coelenterazine luciferase encoded by a synthetic gene. As Lucia luciferase is secreted, it can be directly measured in the cell culture medium using bioluminescent assays.

InvivoGen provides a recombinant Lucia luciferase protein (see Related Products) which is a positive control for QUANTI-Luc™. A dilution series of the recombinant Lucia luciferase protein can also be used to determine the linear range of the assay.

METHODS

Preparation of QUANTI-Luc™
1. Pour the pouch contents into a 50 ml screw cap tube.
2. Add 25 ml of sterile water.
3. Swirl product gently until powder is completely dissolved.
4. Use QUANTI-Luc™ assay solution immediately or store until required for use. Reconstituted QUANTI-Luc™ can be stored for 1 week at 4°C and for 1 month at -20°C. Prepare aliquots to avoid repeated freeze-thaw cycles.

Note: This product is photosensitive and should be protected from light.

Detection of luciferase activity from cell culture medium
To obtain end-point readings using a luminometer with an injector.
1. Set the luminometer with the following parameters: 50 µl of injection, end-point measurement with a 4 second start time and 0.1 second reading time.
2. Pipet 10-20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Prime the injector with the QUANTI-Luc™ assay solution and proceed immediately with the measurement.

To obtain end-point readings using a luminometer without injectors.
1. Set the luminometer with a 0.1 second reading time.
2. Pipet 10-20 µl of sample per well into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Add 50 µl of QUANTI-Luc™ assay solution to each well or tube.
4. Gently tap the plate several times to mix (do not vortex).
5. Proceed immediately with the measurement.

RELATED PRODUCTS

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog Code</th>
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<tbody>
<tr>
<td>QUANTI-Luc™ Gold (for standard and HTS assays)</td>
<td>rep-qleg1</td>
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<tr>
<td>pSelect-zeo-Lucia™ (expression plasmid)</td>
<td>psetz-lucia</td>
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<td>Recombinant Lucia™ protein</td>
<td>rec-lucia</td>
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<td>Reporter Cells</td>
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<tr>
<td>THP1-Dual™ (IRF-Lucia/NF-κB-SEAP) Cells</td>
<td>thpd-nfis</td>
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<tr>
<td>THP1-Lucia™ NF-kB Cells</td>
<td>thpl-nfkb</td>
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For a complete list of InvivoGen’s Lucia luciferase Reporter Cell Lines visit http://www.invivogen.com/lucia-reporter-cells
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

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. Add 1 ml QB reagent and 1 ml QB buffer to 98 ml sterile H₂O.
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.

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:

<table>
<thead>
<tr>
<th></th>
<th>96-well plate</th>
<th>24-well plate</th>
<th>12-well plate</th>
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</thead>
<tbody>
<tr>
<td>QUANTI-Blue™</td>
<td>180 µl</td>
<td>450 µl</td>
<td>900 µl</td>
</tr>
<tr>
<td>Supernatant</td>
<td>20 µl</td>
<td>50 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

TECHNICAL SUPPORT
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InvivoGen
www.invivogen.com
**B. High Throughput Screening procedure**

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.

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**RELATED PRODUCTS**

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<tr>
<td>pNiFty2-SEAP (Zeo+)</td>
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<td>pSELECT-zeo-SEAP</td>
<td>psetz-seap</td>
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<tr>
<td>HEK-Blue™ Detection</td>
<td>hhb-det2</td>
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<tr>
<td>Recombinant SEAP Protein</td>
<td>rec-hseap</td>
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</table>

**Reporters cells**

- HEK-Blue™ hTLR2
- HEK-Blue™ hTLR4
- RAW-Blue™ Cells
- THP1-Blue™ NF-kB Cells
- THP1-Blue™ ISG Cells

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