THP1-Dual™ KO-SAMHD1 Cells
SAMHD1 knockout NF-κB-SEAP & IRF-Lucia luciferase reporter monocytes
Catalog code: thpd-kosamhd1
https://www.invivogen.com/thpd-kosamhd1
For research use only
Version 22F17-AK

PRODUCT INFORMATION

Contents
• 3-7 x 10^6 THP1-Dual™ KO-SAMHD1 cells in a cryovial or shipping flask. IMPORTANT: If cells provided in a cryovial are not frozen upon arrival, contact InvivoGen immediately.
• 1 ml of Blasticidin (10 mg/ml). Store at 4°C or -20 °C.*
• 1 ml of Zeocin® (100 mg/ml). 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 pouch of QUANTI-Luc™ (Lucia luciferase detection reagent). Store pouch at -20°C. Reconstituted QUANTI-Luc™ is stable for 1 week at 4°C and for 1 month at -20°C. Protect from light.
• 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.

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
• Biallelic SAMHD1 gene knockout has been verified by PCR, Western Blot, DNA sequencing and functional assays.
• The stability for 20 passages, following thawing, has been verified.
• These cells are guaranteed mycoplasma-free.

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.

BACKGROUND
SAMHD1 (sterile alpha motif and histidine aspartate domain-containing protein 1) is a nuclear enzyme playing a major role in nucleotide homeostasis. Additionally, it facilitates the replication fork progression and prevents DNA damage. Moreover, SAMHD1 can interfere with mediators of NF-κB and IRF signaling pathways, thus preventing an excessive antiviral and proinflammatory response.

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 FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 100 μg/ml Normocin™

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.*

- **Test Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% (v/v) heat-inactivated FBS, 100 μg/ml penicillin, 100 μg/ml streptomycin
- **Freezing Medium:** 95% FBS and 5% DMSO

Required Selection 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.

3. Transfer the cells to a larger tube containing 15 ml of pre-warmed growth medium (with 20% heat-inactivated FBS).
4. Transfer the cells to a T-25 culture flask containing 5 ml of growth medium (with 20% heat-inactivated FBS).
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium (with 20% heat-inactivated FBS).
6. Transfer the cells to a T-75 culture flask containing 5 ml of growth medium (with 20% heat-inactivated FBS).
7. Place the culture at 37 °C in 5% CO₂.

Frozen Stock Preparation
1. Resuspend cells at a density of 5-7 x 10⁶ cells/ml in freezing medium freshly prepared with cold FBS.

*Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.*
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. Transfer vials to liquid nitrogen for long term storage.

Cell Maintenance
1. After cells have recovered and are growing well (following at least two passages), maintain and subculture the cells in growth medium. To maintain selection pressure, add 10 μg/ml Blasticidin and 100 μg/ml Zeocin® to the growth medium every other passage.
2. Pass the cells every 3 days by inoculating 5 x 10⁵ cells/ml. Do not allow the cell concentration to exceed 2 x 10⁶ cells/ml.

**Optional: PMA induced THP1 Differentiation**
Following Phorbol 12-myristate 13-acetate (PMA) treatment, THP1-Dual™KO-SAMHD1 cells are more sensitive to IRF-inducers, such as LPS i.e. TLR4-dependent IRF activation.

*Note: PMA treatment may activate NF-κB, and thus, lead to higher background readings with QUANTI-Blue™ Solution.*

1. Add 180 μl of THP1-Dual™KO-SAMHD1 cell suspension per well of a 96-well plate (~ 200,000 cells/well).
2. Add 20 μl of PMA (final concentration 10 ng/ml) to the cells for 3 hours at 37°C in 5% CO₂.
3. Wash cells gently with pre-warmed PBS and add 200 μl of pre-warmed growth medium.
4. Incubate for 3 days at 37°C in 5% CO₂.
5. Wash cells with pre-warmed PBS and add 180 μl of growth medium.
6. Proceed with the reporter assay as described below.

**REPORTER ASSAY**
**Cell preparation**
1. Centrifuge at 150 x g (RCF) for 10 min or 300 x g (RCF) for 5 min.
2. Remove supernatant and resuspend THP1-Dual™KO-SAMHD1 cells at 5 x 10⁵ cells/ml in freshly prepared, pre-warmed test medium.

**Stimulation of THP1-Dual™KO-SAMHD1 cells**
1. Add 20 μl of test compound per well of a flat-bottom 96-well plate. Include a NF-κB positive control (e.g. recombinant hIFN-γ at 3 ng/ml), an IRF positive control (e.g. recombinant hIFN-β at 1000 IU/ml), and a negative control (e.g. endotoxin free water)

*Note: Use new tips for each well to avoid cross-contamination.*
2. Add 180 μl of cell suspension (~100,000 cells) per well.
3. Incubate the plate for 18-24 h at 37°C, 5% CO₂.

**Detection of NF-κB induction**
1. Prepare QUANTI-Blue™ Solution following the instructions on the data sheet.
2. Dispense 180 μl of QUANTI-Blue™ Solution per well of a newly flat-bottom 96-well plate.
3. Add 20 μl of stimulated THP1-Dual™KO-SAMHD1 supernatant/well.
4. Incubate the plate at 37°C for 1-3 h.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

**Detection of IRF 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. Prepare QUANTI-Luc™ following the instructions on the data sheet.
2. 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.
3. Add 10 μl of stimulated THP1-Dual™KO-SAMHD1 supernatant per well into a 96-well white (opaque) or black plate, or a luminometer tube.
4. Prime the injector with the QUANTI-Luc™ and proceed with the measurement.

**RELATED PRODUCTS**

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<thead>
<tr>
<th>Product</th>
<th>Cat. Code</th>
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<td>THP1-Dual™KO-DNase cells</td>
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<td>Zeocin®</td>
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<td>PMA</td>
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**QUANTI-Luc™**
A coelenterazine-based luminescence assay reagent
Catalog code: rep-qlc1, rep-qlc2
https://www.invivogen.com/quanti-luc
For research use only
Version 19A04-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 quantitively 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.

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<tr>
<td>QUANTI-Luc™ Gold (For standard and HTS assays)</td>
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<td>pSelect-zeo-Lucia™ (expression plasmid)</td>
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<td>Recombinant Lucia luciferase protein</td>
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<td>Reporter Cells</td>
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<td>THP1-Dual™ (IRF-Lucia/NSxB-SEAP) Cells</td>
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<td>THP1-Lucia™ NSxB Cells</td>
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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 20C16-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. If this occurs, vortex the product 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 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 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 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

Prepare QUANTI-Blue™ Solution Add supernatant to detection reagent Incubate at 37°C for 15 min to 6 h Measure OD using a microplate reader

1. In a sterile bottle or flask, prepare QUANTI-Blue™ Solution by adding:
   - 1 ml of QB reagent and 1 ml of QB buffer to 98 ml of sterile water.
   - 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.

For different cell culture plate formats, please refer to the table below:

<table>
<thead>
<tr>
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<tr>
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<td>50 μl</td>
<td>100 μl</td>
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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:
   - 1 ml of QB reagent and 1 ml of QB buffer to 98 ml of sterile water.
   - 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.

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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 H2O OR  
   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

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