THP1-XBlue™ Cells

NF-κB/AP-1- Reporter Monocytes

Catalog code: thpx-sp

http://www.invivogen.com/thp1-xblue-cell

For research use only

Version 18D06-MM

PRODUCT INFORMATION

Contents and Storage

• 1 vial of THP1-XBlue™ 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 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.

• 10⁶ cells HKLM (Heat killed Listeria monocytogenes; positive control for TLR2 activity). Store at 4°C. Reconstituted HKLM is stable for 1 month at 4°C or for 6 months at -20°C.

• 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. THP1-XBlue™ cells should not be passaged more than 20 times to remain fully efficient. THP1-XBlue™ cells should be maintained in growth medium supplemented with the selective antibiotic, Zeocin™ (200 µg/ml) following every other passage.

Quality Control

• TLR expression was determined by RT-PCR. All TLR mRNAs were detected.

• THP1-XBlue™ cells have been stimulated with various TLR and NOD agonists. Responses were observed in response only to ligands for TLR2, TLR1/2, TLR2/6, TLR4, TLR5, TLR8, NOD1 and NOD2.

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

• THP1-XBlue™ cells are guaranteed mycoplasma-free.

PRODUCT DESCRIPTION

THP1-XBlue™ cells derive from the human monocytic THP-1 cell line. THP1-XBlue™ cells stably express an NF-κB- and AP-1-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. Upon TLR stimulation, THP1-XBlue™ cells activate transcription factors and subsequently the secretion of SEAP which is easily detectable when using QUANTI-Blue™ Solution, a medium that turns purple/blue in the presence of SEAP. THP1-XBlue™ cells respond to multiple to pattern recognition receptor (PRR) agonists that trigger the NF-κB pathway (see figure 1). Considering the concentration of ligands used to stimulate these cells, TLR2, TLR1/2 and TLR2/6 responses are considered to be very strong. TLR5 and TLR8 responses are robust. TLR4 response is rather weak. Responses to TLR3, TLR7 and TLR9 are hardly detectable even when high concentrations of the cognate ligands are used. THP1-XBlue™ cells are resistant to the selectable marker Zeocin™.

Figure 1: TLR and NOD stimulation profile in THP1-XBlue™ and THP1-XBlue™-CD14. Cells were incubated with 1 ng/ml Pam3CSK4 (TLR1/2), 10 ng/ml FSL-1 (TLR2/6), 10⁶ cells/ml HKLM (TLR2), 10 µg/ml poly(I:C) (TLR3), 10 ng/ml LPS-EK (TLR4), 10 ng/ml RecFla-ST (TLR5), 5 µg/ml imiquimod (TLR7), 5 µg/ml CL075 (TLR8), 10 µg/ml ODN2006 (TLR9), 100 ng/ml C12-iEDAP (NOD1) or 1 µg/ml MDP (NOD2). After 24h incubation, TLR/NOD stimulation was assessed by measuring the levels of SEAP in the supernatant by using QUANTI-Blue™.

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.
SAFETY CONSIDERATIONS
Biosafety Level 1

HANDLING PROCEDURES
Required Cell Culture Medium
- Growth Medium: RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated fetal bovine serum (30 min at 56°C), 100 μg/ml Normocin™, Pen-Strep (100 U/ml-100 μg/ml)
- 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.

- 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)

Required Selective Antibiotic
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. After cells have recovered and are growing well (after at least two passages), maintain and subculture the cells in growth medium. To maintain selection pressure, add 200 μg/ml of Zeocin™ to the growth medium every other passage.
2. Pass the cells every 3 days by inoculating 5 x 10⁶ cells/ml. Do not allow the cell concentration to exceed 2 x 10⁶ cells/ml.

Cell maintenance
1. After cells have recovered and are growing well (after at least two passages), maintain and subculture the cells in growth medium. To maintain selection pressure, add 200 μg/ml of Zeocin™ to the growth medium every other passage.
2. Pass the cells every 3 days by inoculating 5 x 10⁶ cells/ml. Do not allow the cell concentration to exceed 2 x 10⁶ cells/ml.

Cell Handling Recommendations
To ensure the best results:
- Use THP1-XBlue™ cells with less than 20 passages.
- Handling of cells should be as short as possible to prevent any damage resulting from the prolonged stay at room temperature without 5% CO₂.

Positive control preparation (HKLM)
1. Prepare a 10⁶ cells/ml solution of HKLM by adding 1 ml of sterile water to the content of the tube.
2. Mix vigorously by vortexing.

Sample preparation
1. Resuspend all powdered samples in endotoxin-free water to avoid activation of TLR4 of the THP-1 cell line.
2. Warm the samples at 37°C before use.
Notes:
- Avoid testing of pure samples soluble only in ethanol or DMSO: these solutions are toxic to the cell line and can result in false negative results.
- We recommend to ensure the absence of cytotoxicity of the sample on cells before running TLR activity detection test. If no cytotoxic effect is observed, the samples should be diluted in endotoxin-free water before testing.

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. Transfer vials to liquid nitrogen for long term storage.
Note: If properly stored, cells should remain stable for years.

Cell maintenance
1. After cells have recovered and are growing well (after at least two passages), maintain and subculture the cells in growth medium. To maintain selection pressure, add 200 μg/ml of Zeocin™ to the growth medium every other passage.
2. Pass the cells every 3 days by inoculating 5 x 10⁶ cells/ml. Do not allow the cell concentration to exceed 2 x 10⁶ cells/ml.

Sample preparation
1. Resuspend all powdered samples in endotoxin-free water to avoid activation of TLR4 of the THP-1 cell line.
2. Warm the samples at 37°C before use.
Notes:
- Avoid testing of pure samples soluble only in ethanol or DMSO: these solutions are toxic to the cell line and can result in false negative results.
- We recommend to ensure the absence of cytotoxicity of the sample on cells before running TLR activity detection test. If no cytotoxic effect is observed, the samples should be diluted in endotoxin-free water before testing.

- Samples containing a phosphatase activity cannot be tested as they can result in false positive results (like serum not previously heat-inactivated).

Reporter Assay
Day 1:
1. Centrifuge cells at 1000-1500 RPM (RCF 200-300 g) for 5 minutes.
2. Remove supernatant and resuspend THP1-XBlue cells at 1 x 10⁶ cells/ml in fresh, pre-warmed growth medium.
3. Add 20 µl of sample per well including HKLM as the positive control and endotoxin free water as a negative control (use new tips for each well to avoid cross-contamination).
4. Add 180 µl of cell suspension (~200,000 cells) per well of a flat-bottom 96-well plate.
5. Incubate the plate at 37°C in a CO₂ incubator for 18-24 h.

Day 2:
6. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed product data sheet.
7. Add 180 µl of resuspended QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
8. Add 20 µl of THP1-XBlue™ cells supernatant.
9. Incubate the plate at 37°C incubator for 1-8 h.
10. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

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<tr>
<th>Product</th>
<th>Description</th>
<th>Cat. Code</th>
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<td>HKLM</td>
<td>TLR2 ligand</td>
<td>thrl-hklm</td>
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<td>Normocin™</td>
<td>Antimicrobial agent</td>
<td>ant-nr-1</td>
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<td>QUANTI-Blue™</td>
<td>SEAP detection medium</td>
<td>rep-qbs1</td>
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<tr>
<td>Zeocin™</td>
<td>Selective antibiotic</td>
<td>ant-zn-1</td>
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</table>
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/quant-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 when not in use.
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>
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<tr>
<th></th>
<th>96-well plate</th>
<th>24-well plate</th>
<th>12-well plate</th>
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<tr>
<td>QUANTI-Blue™</td>
<td>180 µl</td>
<td>450 µl</td>
<td>900 µl</td>
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<tr>
<td>Supernatant</td>
<td>20 µl</td>
<td>50 µl</td>
<td>100 µl</td>
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**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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For a complete list of InvivoGen’s Reporter Cell Lines visit [http://www.invivogen.com/reporter-cells](http://www.invivogen.com/reporter-cells)

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