# THP1-Blue™ ISG Cells

Interferon regulatory factor-inducible SEAP Reporter Monocytes

Catalog code: thp-isg

https://www.invivogen.com/thp1-blue-isg

For research use only

Version 23E30-MM

## PRODUCT INFORMATION

### Contents and Storage

- **3-7 x $10^6$ of THP1-Blue™ ISG 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 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.

- **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 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 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-Blue™ ISG cells should not be passaged more than 20 times to remain fully efficient. THP1-Blue™ ISG cells should be maintained in growth medium supplemented with the selective antibiotic, Zeocin®, following every other passage.

### Quality Control

- Reporter activity validated by functional assays. THP1-Blue™ ISG cells produce SEAP following stimulation with pattern recognition receptor (PRR) agonists that trigger the IFN signaling pathway, such as lipopolysaccharide (LPS) and transfected double-stranded nucleic acid.
- The stability for 20 passages following thawing has been verified.
- THP1-Blue™ ISG 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.

## PRODUCT DESCRIPTION

THP1-Blue™ ISG (interferon-stimulated genes) cells were specifically designed to monitor the interferon (IFN) signaling pathway in a physiologically relevant cell line. They derive from the human THP-1 monocyte cell line by stable integration of an IFN regulatory factor (IRF)-inducible SEAP reporter construct. THP1-Blue™ ISG cells express a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of an ISG54 minimal promoter in conjunction with five IFN-stimulated response elements. As a result, THP1-Blue™ ISG cells allow the monitoring of IRF activation by determining the activity of SEAP. The levels of IRF-induced SEAP in the cell culture supernatant are readily assessed with QUANTI-Blue™ Solution, a SEAP detection reagent.

THP1-Blue™ ISG cells are highly responsive to pathogen recognition receptor (PRR) agonists that trigger the IFN signaling pathway, such as LPS and transfected double-stranded nucleic acid. The differentiation of THP-1 from monocytes to macrophages can be induced with Phorbol 12-myristate 13-acetate (PMA). PMA treatment of THP1 cells induces gene upregulation and a more IFN-specific response[1, 2].

THP1-Blue™ ISG cells are resistant to Zeocin®.

1. Weiden M. *et al.*, 2000. Differentiation of monocytes to macrophages switches the *Mycobacterium tuberculosis* effect on HIV-1 replication from stimulation to inhibition: modulation of interferon response and CCAAT/enhancer binding protein beta expression. J Immunol. 165(4):2028-39. 2. Park EK. *et al.*, 2007. Optimized THP-1 differentiation is required for the detection of responses to weak stimuli. Inflamm Res. 56(1):45-50.

## 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)

**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.*

- **Freezing Medium:** 95% fetal bovine serum (FBS), 5% 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®

TECHNICAL SUPPORT
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### 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 150 x g (RCF) for 10 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 $cm^2$ tissue culture flask containing 5 ml of growth medium (with 20% heat-inactivated FBS).
7. Place the culture at 37°C, 5% $CO_2$.

### Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x $10^6$ 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 (after at least two passages), maintain and subculture the cells in growth medium. To maintain selection pressure, add 100 µg/ml of Zeocin® to the growth medium every other passage.
2. Pass the cells every 3 days by inoculating 5 x $10^5$ cells/ml. Do not allow the cell concentration to exceed 2 x $10^6$ cells/ml.

*Note: To ensure the best results:*

***- Use THP1-Blue™ ISG 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% $CO_2$.*

## PMA-INDUCED DIFFERENTIATION (OPTIONAL)

Following Phorbol 12-myristate 13-acetate (PMA) treatment, THP-1 ISG cells are more sensitive to IFN-inducers, such as transfected Poly(dA:dT), while the response to TLR ligands, such as Pam3CSK4, is diminished.

### Day 1

1. Add 180 µl of THP-1 cell suspension per well of a 96-well plate (~ 100,000 cells/well).
2. Treat cells with 20 µl of PMA (final concentration 20-50 ng/ml) for 3 hours at 37°C, 5% $CO_2$.
3. Wash cells gently with pre-warmed PBS and add 200 µl pre-warmed growth medium.

### Day 4

4. Wash cells with pre-warmed PBS and add 180 µl growth medium.
5. Add 20 µl of an IFN inducer, such as Poly(dA:dT)/LyoVec™.
6. Incubate overnight at 37°C in 5% $CO_2$.

## DETECTION OF IFN INDUCTION

### 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.*

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

### IFN Induction

1. Centrifuge cells at 150 x g (RCF) for 10 minutes or at 300 x g (RCF) for 5 minutes.
2. Remove supernatant and resuspend THP1-Blue™ ISG cells at 5 x $10^5$ cells/ml in fresh, pre-warmed growth medium.
3. Add 20 µl of sample per well including Poly(dA:dT)/LyoVec™ 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 (~100,000 cells) per well of a flat-bottom 96-well plate.
5. Incubate the plate at 37°C in a $CO_2$ incubator for 18-24 h.
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-Blue™ ISG 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

| Product | Description | Cat. Code |
|---|---|---|
| Normocin™ | Antimicrobial agent | ant-nr-1 |
| Pam3CSK4 | TLR2 ligand | tlrl-pms |
| PMA | Phorbol myristate acetate | tlrl-pma |
| Poly(dA:dT)/LyoVec™ | RIG-I & CDS ligand | tlrl-patc |
| Poly(I:C) HMW/LyoVec™ | RIG-I/MDA-5 ligand | tlrl-piclv |
| QUANTI-Blue™ Solution | SEAP detection reagent | rep-qbs1 |
| Zeocin® | Selection antibiotic | 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, 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

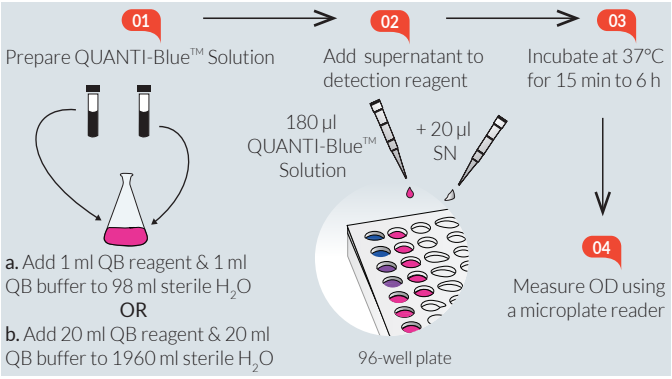


Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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

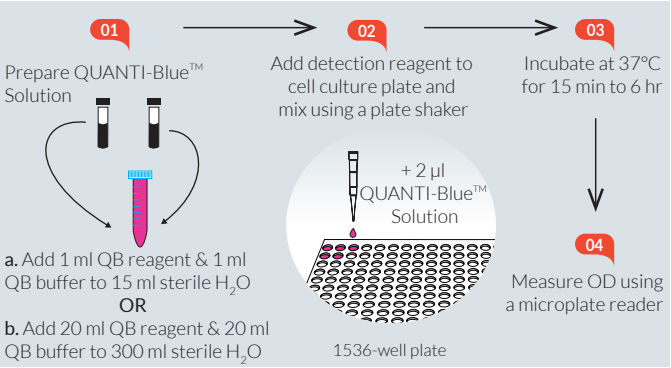


**Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.**

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