

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:** 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

Blasticidin

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. 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 10 µg/ml of blasticidin 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.

Note: To ensure the best results:

- Use THP1-Blue™ NF-κB 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.

DETECTION OF TLR STIMULATION

Positive Control Preparation (HKLM)

1. Prepare a 10⁹ 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 a 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).

TLR Assay

1. Centrifuge cells at 1000-1500 RPM (RCF 200 - 300 g) for 5 minutes.

2. Remove supernatant and resuspend THP1-Blue™ NF-κB cells at 5 x 10⁵ 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 (~100,000 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.

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™ NF-κB 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
Blasticidin	Selective antibiotic	ant-bl-1
CL075	TLR8 ligand	tlrl-c75
FLA-ST Ultrapure	TLR5 ligand	tlrl-pstfla
FSL-1	TLR2 ligand	tlrl-fsl
HKLM	TLR2 ligand	tlrl-hklm
LPS-EB Ultrapure	TLR4 ligand	tlrl-3pelps
MDP	NOD2 ligand	tlrl-mdp
Normocin™	Antimicrobial agent	ant-nr-1
Pam3CSK4	TLR2 ligand	tlrl-pms
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs1
Tri-DAP	NOD1 ligand	tlrl-tdap

TECHNICAL SUPPORT

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Any questions about our cell lines?
Visit our FAQ page.

 **InvivoGen**
www.invivogen.com

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

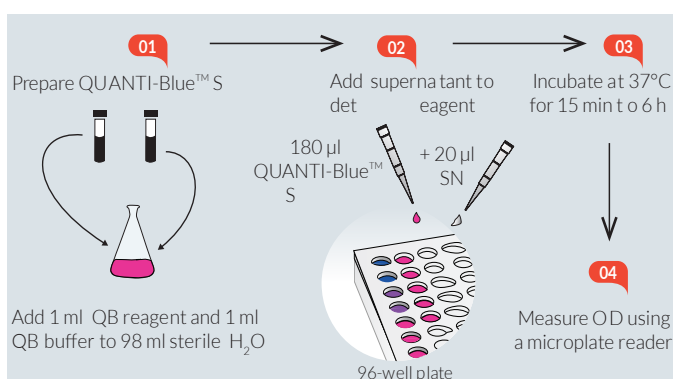


Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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 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:

	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 procedure

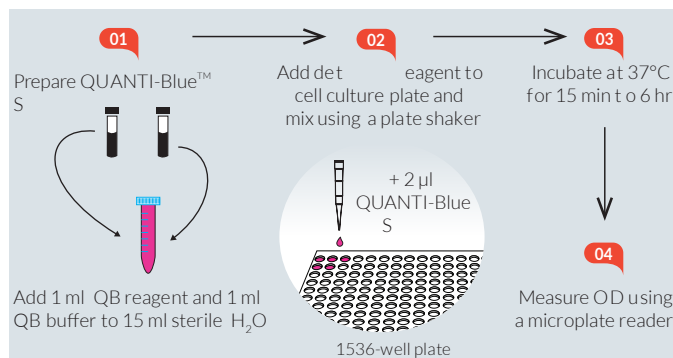


Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

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.

RELATED PRODUCTS

Product	Catalog Code
pNiFty2-SEAP (Zeo [®])	pnifty2-seap
pSELECT-zeo-SEAP	psetz-seap
HEK-Blue™ Detection Recombinant SEAP Protein	hb-det2 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 <http://www.invivogen.com/reporter-cells>

TECHNICAL SUPPORT

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