THP1-Dual™ Cells

NF-κB-SEAP and IRF-Lucia luciferase Reporter Monocytes

Catalog code: thpd-nfis

http://www.invivogen.com/thp1-dual

For research use only

Version 18D04-MM

PRODUCT INFORMATION

Contents and Storage

- **1 vial of THP1-Dual™ cells** (3-7 x 10^6 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), 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). 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.

Quality Control

- Reporter activity has been validated using functional assays.
- The stability of this cell line for 20 passages following thawing has been verified.
- THP1-Dual™ 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 to 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-Dual™ cells were derived from the human THP-1 monocyte cell line by stable integration of two inducible reporter constructs. THP1-Dual™ cells feature the Lucia luciferase gene, a new secreted luciferase reporter gene, under the control of an ISG54 (interferon-stimulated gene) minimal promoter in conjunction with five interferon (IFN)-stimulated response elements. THP1-Dual™ cells also express a secreted embryonic alkaline phosphatase (SEAP) reporter gene driven by an IFN-β minimal promoter fused to five copies of the NF-κB consensus transcriptional response element and three copies of the c-Rel binding site.

As a result, THP1-Dual™ cells allow the simultaneous study of the NF-κB pathway, by monitoring the activity of SEAP, and the interferon regulatory factor (IRF) pathway, by assessing the activity of Lucia luciferase. Both reporter proteins are readily measurable in the cell culture supernatant when using QUANTI-Blue™, a SEAP detection reagent, and QUANTI-Luc™, a Lucia luciferase detection reagent. THP1-Dual™ cells induce the activation of NF-κB in response to certain TLR agonists, such as Pam3CSK4 and flagellin. They trigger the IRF pathway upon stimulation with type I IFNs and RLR (RIG-I-like receptor), CDS (cytosolic dsDNA sensor) or STING agonists, such as transfected poly(I:C) or poly(dA:dT) and 2'3'-cGAMP respectively.

THP1-Dual™ cells are resistant to the selectable markers 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% 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 Antibiotics

Blasticidin and 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 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 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 10 µg/ml of blasticidin and 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-Dual™ 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.

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.

Optional: PMA-induced THP-1 Differentiation

Following Phorbol 12-myristate 13-acetate (PMA) treatment, THP1-Dual™ cells are more sensitive to IFN-inducers, such as transfected Poly(dA:dT), while the response to certain TLR ligands, such as Pam3CSK4, is diminished.

Note: PMA treatment may activate NF-κB, leading to higher background readings when used to detect TLR stimulation.

Day 1

1. Add 180 µl of THP1-Dual™ 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 of pre-warmed growth medium.

Day 4

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

CELL PREPARATION

1. Centrifuge cells at 1000-1500 RPM (RCF 200-300 g) for 5 minutes.
2. Remove supernatant and resuspend THP1-Dual™ cells at 5 x 10^5 cells/ml in freshly prepared test medium.

DETECTION OF TLR STIMULATION

1. Add 20 µl of sample per well including a positive control (such as Pam3CSK4) 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% CO_2.
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™ 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 kinetic measurements or a luminometer with a manual set-up.

1. 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).
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% CO_2.
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™ 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

Product	Description	Cat. Code
5'ppp-dsRNA/LyoVec™	RIG-I ligand	tlrl-3prnalv
Blasticidin	Selective antibiotic	ant-bl-1
FLA-ST Ultrapure	TLR5 ligand	tlrl-epstfla
LPS-EB Ultrapure	TLR4 ligand	tlrl-3pelps
LyoVec™	Transfection reagent	lyec-12
Normocin™	Antimicrobial agent	ant-nr-1
Pam3CSK4	TLR2 ligand	tlrl-pms
Poly(dA:dT)/LyoVec™	RIG-I & CDS ligand	tlrl-patc
Poly(I:C) HMW/LyoVec™	RIG-I/MDA-5 ligand	tlrl-piclv
PMA	Phorbol 12-myrisate 13-acetate	tlrl-pma
QUANTI-Blue™ Solution	SEAP detection medium	rep-qbs1
QUANTI-Luc™	Luminesence assay reagent	rep-qlc1
R848	TLR7/8 ligand	tlrl-r848
Zeocin™	Selective antibiotic	ant-zn-1

TECHNICAL SUPPORT
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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 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.

RELATED PRODUCTS

Product	Catalog Code
QUANTI-Luc™ Gold (For standard and HTS assays)	rep-qlcg1
pSelect-zeo-Lucia™ (expression plasmid)	psetz-lucia
Recombinant Lucia™ protein	rec-lucia
Reporter Cells	
THP1-Dual™ (IRF-Lucia/NF-κB-SEAP) Cells	thpd-nfis
THP1-Lucia™ NF-κB Cells	thp1-nfkb

For a complete list of InvivoGen's Lucia luciferase Reporter Cell Lines visit http://www.invivogen.com/lucia-reporter-cells

TECHNICAL SUPPORT
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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

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

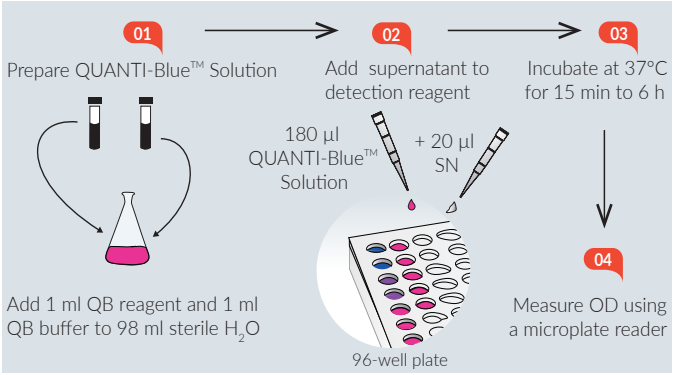

Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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

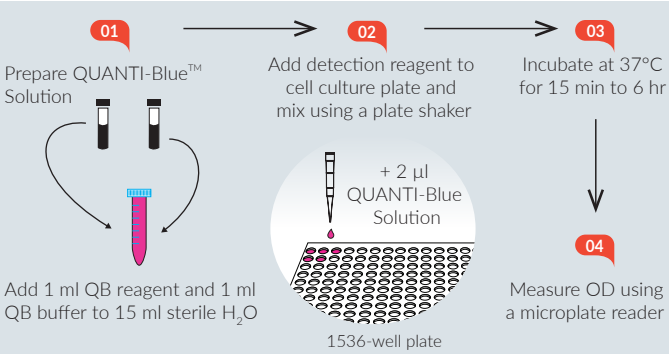

Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

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^R)	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
http://www.invivogen.com/reporter-cells