A549-Dual™ Cells

NF-κB-SEAP & IRF-Lucia reporter lung carcinoma cells

Catalog code: a549d-nfis

https://www.invivogen.com/a549-dual

For research use only

Version 19D16-MM

PRODUCT INFORMATION

Contents

- 1 vial of A549-Dual™ Cells (3-7 x 10^6 cells)
- 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 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.
- 1 pouch of QUANTI-Luc™. Store QUANTI-Luc™ pouch at -20 °C for 12 months. Reconstituted QUANTI-Luc™ medium is stable for 1 week at 4 °C and for 1 month at -20 °C. Protect QUANTI-Luc™ from light.

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. A549-Dual™ cells should not be passaged more than 20 times to remain fully efficient. A549-Dual™ cells should be maintained in growth medium supplemented with two selective antibiotics, blasticidin (10 µg/ml) and Zeocin™ (100 µg/ml).

Quality Control

- For each lot, proper activation of the NF-κB pathway and IRF pathway is confirmed upon stimulation of A549-Dual™ cells by various pathogen associated molecular patterns (PAMPs) known to activate these pathways.
- The stability of this cell line for 20 passages following thawing has been verified.
- A549-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 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

CELL LINE DESCRIPTION

A549-Dual™ cells are adherent epithelial cells that have been derived from the human A549 lung carcinoma cell line by stable integration of two inducible reporter constructs. The A549 cell line is a well-characterized cellular model for asthma, allergies and respiratory infections. A549-Dual™ cells express a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB binding sites. A549-Dual™ cells also express the Lucia luciferase gene, which encodes a secreted luciferase, under the control of an ISG54 minimal promoter in conjunction with five IFN-stimulated response elements. As a result, A549-Dual™ cells allow to simultaneously study the NF-κB pathway, by assessing the activity of SEAP, and the interferon regulatory factor (IRF) pathway, by monitoring 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™ detection reagent.

A549-Dual™ cells express numerous pattern recognition receptors (PRRs), including the RIG-I-like receptor (RLR) RIG-I[1, 2], and the Toll-like receptors (TLRs) TLR2[3], TLR3[4, 5] and TLR5[6] but not TLR4[3]. Upon recognition of their cognate PAMPs, these receptors induce signaling pathways leading to the activation of the transcription factors NF-κB and/or IRF3/7. Stimulation of A549-Dual™ cells with the following PAMPs, Pam3CSK4 (TLR2) Poly(I:C) (TLR3), flagellin (TLR5), leads to the activation of NF-κB. IL-1β or TNF-α can be used as positive controls to activate the NF-κB signaling pathway. Stimulation with RLR ligands, such as transfected poly(I:C) or poly(dA:dT), or the STING agonist, 2'3'-cGAMP, triggers the IRF pathway. IFN-α can be used as a positive control to activate the IRF signaling pathway.

A549-Dual™ cells are resistant to the selectable markers blasticidin and Zeocin™.

1. Kolokoltsova OA. et al., 2014. RIG-I enhanced interferon independent apoptosis upon Junin virus infection. PLoS One. 9(6):e99610. **2. Hagmann CA. et al., 2013.** RIG-I detects triphosphorylated RNA of Listeria monocytogenes during infection in non-immune cells. PLoS One. 8(4):e62872. **3 Slevogt H. et al., 2007.** Moraxella catarrhalis is internalized in respiratory epithelial cells by a trigger-like mechanism and initiates a TLR2- and partly NOD1-dependent inflammatory immune response. Cell Microbiol. 9(3):694-707. **4. Taura M. et al., 2008.** p53 regulates Toll-like receptor 3 expression and function in human epithelial cell lines. Mol Cell Biol. 28(21):6557-67. **5. Tissari J. et al., 2015.** IFN-alpha enhances TLR3-mediated antiviral cytokine expression in human endothelial and epithelial cells by up-regulating TLR3 expression J Immunol. 174(7):4289-94. **6. Tallant T. et al., 2004.** Flagellin acting via TLR5 is the major activator of key signaling pathways leading to NF-kappa B and proinflammatory gene program activation in intestinal epithelial cells. BMC Microbiol. 4:33.

TECHNICAL SUPPORT
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SAFETY CONSIDERATIONS

Biosafety Level 1

HANDLING PROCEDURES

Required Cell Culture Medium

• **Growth Medium:** DMEM, 2 mM L-glutamine, 4.5 g/l glucose, 10% (v/v) fetal bovine serum (FBS), Pen-Strep (100 U/ml-100 µg/ml), 100 µg/ml Normocin™

• **Freezing Medium:** DMEM with 20% (v/v) FBS and 10% (v/v) DMSO

• **Test Medium for use with** QUANTI-Blue™**:** DMEM, 2 mM L-glutamine 4.5 g/l glucose, 10% (v/v) heat-inactivated FBS (30 min at 56 ºC), Pen-Strep (100 U/ml-100 µg/ml), 100 µg/ml Normocin™

Required Selective Antibiotic(s)

• 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.

Note: All steps from this point should be carried out under strict aseptic conditions.

3. Transfer cells in a larger vial containing 15 ml of pre-warmed growth medium. **Do not add selective antibiotics until the cells have been passaged twice.**
4. Centrifuge vial at 1000-1200 RPM (RCF = 200-300 g) for 5 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium without selective antibiotics.
6. Transfer the vial contents to a T-25 culture flask containing 5 ml of growth medium.
7. Place the culture at 37 °C in 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10^6 cells/ml in freezing medium prepared extemporaneously with cold growth medium.

Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.

2. Aliquot 1 ml cells 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 one passage), maintain and subculture the cells in growth medium supplemented with 10 µg/ml of blasticidin and 100 µg/ml of Zeocin™.
2. Renew growth medium twice a week.
3. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency. Rinse cell layer with PBS and detach cells with 0.25% trypsin-EDTA for 2-5 minutes.

Note: To ensure the best results:

- Use A549-Dual™ cells with less than 20 passages after thawing.

- 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.

REPORTER ASSAYS

Note: For best results, 24 hours prior to the test we recommend to prepare a subculture at a 1:3 ratio.

Cell preparation

1. Rinse cell layer with PBS and detach cells with trypsin.
2. Centrifuge cells at 1000-1500 RPM (RCF 200-300 g) for 5 min.
3. Remove supernatant and resuspend A549-Dual™ cells at 2.8 x 10^5 cells/ml in fresh, pre-warmed growth medium.

NF-κB induction

1. Add 20 µl of sample per well of a flat-bottom 96-well plate, including a positive control (e.g. recombinant IL-1β or TNF-α) and endotoxin free water as a negative control.

Note: Use new tips for each well to avoid cross-contamination.

2. Add 180 µl of cell suspension (~50,000 cells) per well.
3. Incubate the plate at 37 °C in a CO_2 incubator for 18-24 h.
4. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed data sheet.
5. Add 20 µl of A549-Dual™ cells supernatant.
6. Add 180 µl of QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
7. Incubate the plate at 37 °C in a CO_2 incubator for 1-8 h.
8. Determine NF-κB-induced SEAP levels using a microplate spectrophotometer at 620-655 nm.

IRF 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 a positive control (e.g. PolydA:dT)/LyoVec™ or recombinant IFN-α) and endotoxin free water as a negative control.

Note: use new tips for each well to avoid cross-contamination.

2. Add 180 µl of cell suspension (~50,000 cells) per well of a flat-bottom 96-well plate.
3. Incubate the plate at 37 °C in a CO_2 incubator for 18-24 h.
4. Prepare the QUANTI-Luc™ assay solution 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 samples (20 µl per well) into a 96-well white (opaque) or black plate, or a luminometer tube.
7. Prime the injector with the assay solution and proceed with the measurement.

RELATED PRODUCTS

Product	Catalog Code
Blasticidin	ant-bl-1
Poly(dA:dT)/LyoVec™	tlrl-patc
Poly(I:C) (HMW)/LyoVec™	tlrl-piclv
5'ppp-dsRNA/LyoVec™	tlrl-3prnalv
QUANTI-Blue™ Solution	rep-qbs
QUANTI-Luc™	rep-qlc1
Recombinant human IL-1β	rcyec-hil1b
Recombinant human TNF-α	rcyc-htnfa
Zeocin™	ant-zn-1

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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

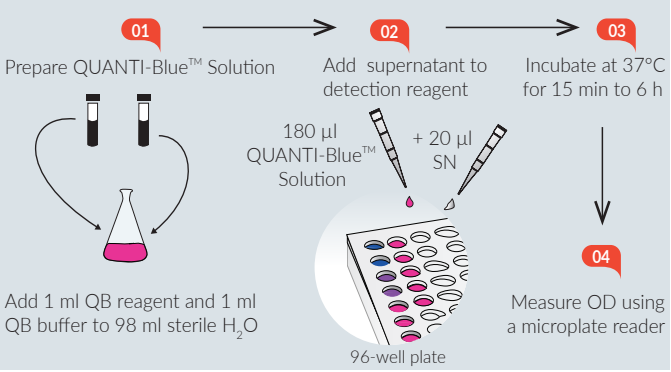

Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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

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B. High Throughput Screening procedure

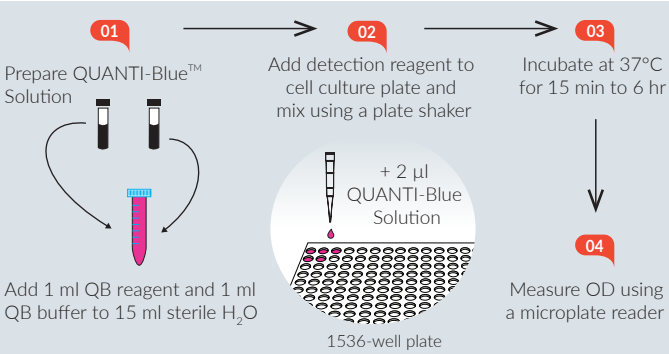

Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

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

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

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