**J774-Dual™ Cells**

**NF-κB-SEAP & IRF-Luc Reporter Macrophages**

Catalog code: j774d-nfis

https://www.invivogen.com/j774-dual

**For research use only**

Version 19E16-MM

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**PRODUCT INFORMATION**

**Contents and Storage**

- **1** vial of J774-Dual™ 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 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 Normocin™ (50 mg/ml), a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store 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 or for 1 month at -20°C. Protect QUANTI-Luc™ 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**

- For each lot, proper activation of the NF-κB pathway and IRF pathway is confirmed upon stimulation of J774-Dual™ cells by various pathogen associated molecular patterns (PAMPs) known to activate these pathways (see validation data available on our website at www.invivogen.com/j774-dual).

- The stability of this cell line for 20 passages following thawing has been verified.

- J774-Dual™ cells are guaranteed mycoplasma-free.

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**PRODUCT DESCRIPTION**

J774-Dual™ cells have been derived from the mouse J774.1 macrophage-like cell line by stable integration of two inducible reporter constructs. J774-Dual™ cells express a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of an IFN-γ minimal promoter fused to five copies of the NF-κB consensus transcriptional response element and three copies of the c-Rel binding site. J774-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, J774-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™ Solution, a SEAP detection reagent, and QUANTI-Luc™, a Lucia luciferase detection reagent.

J774.1 cells express a variety of pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) 1-6, C-type lectin receptors (CLRs) 2-5, RIG-I-like receptors (RLRs). 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 J774-Dual™ cells with the following PAMPs, Pam3CSK4 (TLR1/2), lipopolisaccharide (TLR4), CpG ODNs (TLR9), L18-MDP (NOD2) and TDB (Mincle), leads to the activation of NF-κB. Stimulation with RLR ligands, such as transfected poly(I:C) or 5′ppp-dsRNA, or the STING agonist, 2′,3′-cGAMP, triggers the IRF pathway (see validation data available on our website at www.invivogen.com/j774-dual).

J774-Dual™ cells are resistant to the selectable markers blasticidin and Zeocin™.


**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.

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**TECHNICAL SUPPORT**

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HANDLING PROCEDURES

Required Cell Culture Medium
- Growth Medium: DMEM (2 mM L-glutamine, 3.7 g/L sodium bicarbonate, 4.5 g/L glucose and 1.0 mM sodium pyruvate) with 10% heat-inactivated fetal bovine serum (30 min at 56 °C), 100 µg/ml Normocin™, Pen-Strep (100 U/ml-100 µg/ml)
- Freezing Medium: 90% heat-inactivated fetal bovine serum and 10% DMSO

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 vial containing 15 ml of pre-warmed growth medium.
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. Do not add selective antibiotics.
6. Transfer the vial contents to a 25 cm² tissue culture flask containing 5 ml of growth medium.
7. Place the culture at 37°C in 5% CO₂.
Note: When recovering J774-Dual™ cells, it is not unusual to find a number of non-adherent but viable cells in the initial propagation.

Frozen Stock Preparation
1. Resuspend cells at a density of 5-7 × 10⁴ cells/ml in freezing medium prepared extemporaneously with cold growth medium.
2. Aliquot 1 ml cells into cryovials.
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 5 µg/ml of Blasticidin and 100 µg/ml of Zeocin™.
2. Pass the cells every 3 days using a cell scraper and by inoculating 2.5 x 10⁵ cells/cm². Do not allow the cell concentration to exceed 2 x 10⁵ cells/cm².
Notes:
- To ensure the best results:
  - Use J774-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% CO₂.

REPORTER ASSAYS
Note: For better results, we recommend to inoculate a flask with 2.5 x 10⁵ cells/cm², three days prior to the test.

NK-κB induction
1. As described under HANDLING PROCEDURES.
2. Centrifuge cells at 1000-1500 RPM (RCF 200-300 g) for 5 min.
3. Remove supernatant and resuspend J774-Dual™ cells at 2.8 x 10⁶ cells/ml in fresh, pre-warmed growth medium.
4. Add 20 µl of sample per well of a flat-bottom 96-well plate, including a positive control (e.g. Pam3CSK4) and endotoxin-free water as a negative control.
Note: use new tips for each well to avoid cross-contamination.
5. Add 180 µl of cell suspension (~50,000 cells) per well.
6. Incubate the plate at 37°C in a CO₂ incubator for 18-24 h.
7. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed product data sheet.
8. Add 170 µl of resuspended QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
9. Add 30 µl of J774-Dual™ cells supernatant.
10. Incubate the plate at 37°C in a CO₂ incubator for 1-8 h.
11. 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. Use a cell scraper to detach cells and count the number of cells.
2. Centrifuge cells at 1000-1500 RPM (RCF 200-300 g) for 5 min.
3. Remove supernatant and resuspend J774-Dual™ cells at 2.8 x 10⁵ cells/ml in fresh, pre-warmed growth medium.
4. Add 20 µl of sample per well including a positive control (e.g. 2′,3′-cGAMP) and endotoxin free water as a negative control.
Note: use new tips for each well to avoid cross-contamination.
5. Add 180 µl of cell suspension (~50,000 cells) per well of a flat-bottom 96-well plate.
6. Incubate the plate at 37°C in a CO₂ incubator for 18-24 h.
7. Prepare the QUANTI-Luc™ assay solution following the instructions on the enclosed product data sheet.
8. 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.
9. Pipet samples (20 µl per well) into a 96-well white (opaque) or black plate, or a luminometer tube.
10. Prime the injector with the assay solution and proceed with the measurement.

RELATED PRODUCTS

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<th>Product</th>
<th>Description</th>
<th>Catalog Code</th>
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<td>2′,3′-cGAMP</td>
<td>STING agonist</td>
<td>tlr1-nacga23</td>
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<tr>
<td>Blasticidin</td>
<td>Selection antibiotic</td>
<td>ant-bl-1</td>
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<tr>
<td>Normocin™</td>
<td>Antimicrobial agent</td>
<td>ant-nr-1</td>
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<tr>
<td>Pam3CSK4</td>
<td>TL1/2 agonist</td>
<td>tlr1-pms</td>
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<tr>
<td>QUANTI-Blue™ Solution</td>
<td>SEAP detection reagent</td>
<td>rep-qbs</td>
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<tr>
<td>QUANTI-Luc™</td>
<td>Lucia detection reagent</td>
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<td>Zeocin™</td>
<td>Selection antibiotic</td>
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</table>

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E-mail: info@invivogen.com

www.invivogen.com
QUANTI-Luc™
A coelenterazine-based luminescence assay reagent
Catalog code: rep-qlc1, rep-qlc2
https://www.invivogen.com/quanti-luc
For research use only
Version 19A04-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

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog Code</th>
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<td>QUANTI-Luc™ Gold (For standard and HTS assays)</td>
<td>rep-qlcg1</td>
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<td>pSelect-zeo-Lucia™ (expression plasmid)</td>
<td>psetz-lucia</td>
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<td>Recombinant Lucia luciferase protein</td>
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<td>Reporter Cells</td>
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<td>THP1-Dual™ (IRF-Lucia/NF-κB-SEAP) Cells</td>
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<td>THP1-Lucia™ NF-κB Cells</td>
<td>thp1-nfkb</td>
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</table>

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

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InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com
QUANTI-Blue™ Solution

Medium for detection and quantification of alkaline phosphatase in standard and HTS assays

Catalog code: rep-qbs, rep-qbs2

https://www.invivogen.com/quanti-blue

For research use only

Version 18L10-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. Protect QUANTI-Blue™ 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) assay 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

1. Prepare 100 ml of QUANTI-Blue™ Solution by adding 1 ml of QB reagent and 1 ml QB buffer to 98 ml sterile H2O.
2. Mix well by vortexing and incubate at room temperature for 10 min.
3. Use QUANTI-Blue™ supernatant to 96-well plate.
4. Incubate at 37°C for 15 min to 6 h.
5. Measure optical density (OD) at 620-655 nm using a microplate reader.

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.

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 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 70°C for 30 min to inactivate the alkaline phosphatase activity.

For different cell culture plate formats, please refer to the table below:

<table>
<thead>
<tr>
<th></th>
<th>96-well plate</th>
<th>24-well plate</th>
<th>12-well plate</th>
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<tbody>
<tr>
<td>QUANTI-Blue™</td>
<td>180 µl</td>
<td>450 µl</td>
<td>900 µl</td>
</tr>
<tr>
<td>Supernatant</td>
<td>20 µl</td>
<td>50 µl</td>
<td>100 µl</td>
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</table>

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www.invivogen.com
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 H₂O 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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**RELATED PRODUCTS**

<table>
<thead>
<tr>
<th>Product</th>
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<td>pNiFty2-SEAP (Zeo⁺)</td>
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<td>HEK-Blue™ Detection</td>
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<tr>
<td>THP1-Blue™ ISG Cells</td>
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For a complete list of InvivoGen’s Reporter Cell Lines visit http://www.invivogen.com/reporter-cells

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