Jurkat-Dual™ Cells
IRF-SEAP & NF-kB-Luc Reporter T lymphocytes
Catalog code: jktd-isnf
https://www.invivogen.com/jurkat-dual-cells
For research use only
Version 21A04-MM

PRODUCT INFORMATION
Contents and Storage
- 3-7 x 10^6 Jurkat-Dual™ 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 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), Normocin® is 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.
  - 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. Keep reconstituted QUANTI-Luc™ away from light.
  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’.

Quality Control
- Reporter activity has been validated using functional assays.
- The stability for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

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.

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.
SAFETY CONSIDERATIONS
Biosafety Level 1

HANDLING PROCEDURES
Required Cell Culture Medium
- Growth Medium: IMDM, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated fetal bovine serum (FBS; 30 min at 56 °C), 100 μg/ml Normocin™, Pen-Strep (100 U/ml-100 μg/ml)
  Note: Heat-inactivated FBS is also commercially available.
- Freezing Medium: 90% heat-inactivated FBS and 10% DMSO
- Test Medium: IMDM, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated FBS, Pen-Strep (100 U/ml-100 μg/ml) without Normocin, Blasticidin, and Zeocin™

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 cells at 150 x g (RCF) for 10 mins.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium.
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.

Frozen Stock Preparation
1. Resuspend cells at a density of 5-7 x 10⁴ cells/ml in freshly prepared freezing medium made with cold FBS.
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 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 10⁵ cells/ml. Do not allow the cell concentration to exceed 2 x 10⁶ cells/ml.
  Note: To ensure the best results:
- Use Jurkat-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% CO₂.

REPORTER ASSAYS
IRF induction
1. Centrifuge cells at 800 RPM (RCF 150 g) for 5 minutes.
2. Remove supernatant and resuspend Jurkat-Dual™ cells at 2 x 10⁴ cells/ml in fresh, pre-warmed test medium.
3. Add 20 μl of sample per well including Poly(I:C) 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 (~400,000 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 20 μl of Jurkat-Dual™ cells supernatant.
8. Add 180 μl of resuspended QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
9. Incubate the plate at 37°C incubator for 1-8 h.
10. Determine SEAP levels using a spectrophotometer at 620-655 nm.

NK-κB 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. Centrifuge at 150 x g (RCF) for 10 mins or 300 x g (RCF) for 5 mins.
2. Remove supernatant and resuspend Jurkat-Dual™ cells at 2 x 10⁵ cells/ml in fresh, pre-warmed test medium.
3. Add 20 μl of sample per well including concanavalin A 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 (~400,000 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 the QUANTI-Luc™ assay solution following the instructions on the enclosed product data sheet.
7. 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.
8. Pipet samples (10 μl per well) into a 96-well white (opaque) or black plate, or a luminometer tube.
9. Prime the injector with the assay solution and proceed with the measurement.

RELATED PRODUCTS

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Cat. Code</th>
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<tbody>
<tr>
<td>Blasticidin</td>
<td>Selective antibiotic</td>
<td>ant-bl-1</td>
</tr>
<tr>
<td>Normocin™</td>
<td>Antimicrobial agent</td>
<td>nt-nr-1</td>
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<tr>
<td>Poly(I:C)</td>
<td>Synthetic analog of dsRNA</td>
<td>tlr-l-pc</td>
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<td>QUANTI-Blue™ Solution</td>
<td>SEAP detection medium</td>
<td>rep-qbs</td>
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<tr>
<td>QUANTI-Luc™</td>
<td>Luciferase detection reagent</td>
<td>rep-qlc1</td>
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<tr>
<td>Zeocin™</td>
<td>Selective antibiotic</td>
<td>ant-zn-1</td>
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TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com

Any questions about our cell lines?
Visit our FAQ page.
**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](https://www.invivogen.com/quanti-blue)

For research use only

Version 20C16-MM

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**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.

**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.**

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**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.

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**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.](https://www.invivogen.com/community/figure.png)

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:
   - 1 ml of QB reagent and 1 ml of QB buffer to 98 ml of sterile water.
   - 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.

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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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</thead>
<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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**TECHNICAL SUPPORT**

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

www.invivogen.com
B. High Throughput Screening (HTS) procedure

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 QB reagent and 1 ml QB buffer to 15 ml sterile H2O OR
   b. 20 ml QB reagent and 20 ml QB buffer to 300 ml sterile H2O
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

<table>
<thead>
<tr>
<th>Product</th>
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<td>pNiFty2-SEAP (Zeo+)</td>
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<td>pSELECT-zeo-SEAP</td>
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<td>HEK-Blue™ Detection</td>
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<td>Reporter cells</td>
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<td>HEK-Blue™ hTLR4</td>
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<td>raw-sp</td>
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<td>THP1-Blue™ NF-κB Cells</td>
<td>thp-nfkb</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 https://www.invivogen.com/reporter-cells
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.

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<table>
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<th>Product</th>
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<td>QUANTI-Luc™ Gold (For standard and HTS assays)</td>
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<tr>
<td>pSelect-zeo-Lucia™ (expression plasmid)</td>
<td>psetz-lucia</td>
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<tr>
<td>Recombinant Lucia luciferase protein</td>
<td>rec-lucia</td>
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<td>Reporter Cells</td>
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<td>THP1-Dual™ (IRF-Lucia/NF-κB-SEAP) Cells</td>
<td>thpd-nfis</td>
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<tr>
<td>THP1-Lucia™ NF-κB Cells</td>
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