Jurkat-Dual™ Cells
IRF-SEAP & NF-κB-Luc Reporter T lymphocytes
Catalog code: jktd-isnf
https://www.invivogen.com/jurkat-dual-cells
For research use only
Version 19K04-MM

PRODUCT INFORMATION
Contents and Storage
• 1 vial of Jurkat-Dual™ cells (3-7 x 10^6 cells)

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). Normocin™ is a formulation of three antibiotics active against mycoplasma, 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 Cells Upon Receipt
Cells must be thawed immediately upon receipt and grown according to handling procedures (as described on the next page), to ensure cell viability and proper assay performance.

Note: Do not freeze the 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.

Quality Control
• Reporter activity has been validated using functional assays.
• The stability of this cell line 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.

PRODUCT DESCRIPTION
Jurkat-Dual™ cells were derived from the human T lymphocyte-based Jurkat cell line by stable integration of two inducible reporter constructs. Jurkat-Dual™ cells feature the Lucia™ gene, a new secreted luciferase 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. Jurkat-Dual™ cells also express a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of an ISG54 minimal promoter in conjunction with five IFN-stimulated response elements. As a result, Jurkat-Dual™ cells allow to study simultaneously the NF-κB pathway, by monitoring the activity of Lucia™, and the interferon regulatory factor (IRF) pathway, by assessing the activity of SEAP. Both reporter proteins are readily measurable in the cell culture supernatant using QUANTI-Luc™ or QUANTI-Blue™, respectively.

Jurkat-Dual™ cells were incubated with 50 μg/ml phytohemagglutinin (PHA), 50 μg/ml concanavalin A (Con A), IFN-κBα, 200 ng/ml TNF-α or 10 μg/ml poly(I:C). After 24h incubation, the levels of NF-κB-induced Lucia and IRF-induced SEAP were assessed from the cell culture supernatant using QUANTI-Luc™ or QUANTI-Blue™, respectively.

Figure 1: NF-κB/IRF dual response of Jurkat-Dual™ cells. Cells were incubated with 50 μg/ml phytohemagglutinin (PHA), 50 μg/ml concanavalin A (Con A), 10 μg/ml IFN-κBα, 200 ng/ml TNF-α or 10 μg/ml poly(I:C). After 24h incubation, the levels of NF-κB-induced Lucia and IRF-induced SEAP were assessed from the cell culture supernatant using QUANTI-Luc™ or QUANTI-Blue™, respectively.
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-orn 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 800 RPM (RCF 150 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 vials 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 10⁶ 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 10⁶ 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 cells at 800 RPM (RCF 150 g) for 5 minutes.
2. Remove supernatant and resuspend Jurkat-Dual™ cells at 2 x 10⁶ 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

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<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>
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<tr>
<td>Normocin™</td>
<td>Antimicrobial agent</td>
<td>rt-nr-1</td>
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<td>Poly(I:C)</td>
<td>Synthetic analog of dsRNA</td>
<td>tlr-l-pic</td>
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<td>QUANTI-Blue™</td>
<td>SEAP detection medium</td>
<td>rep-qbs</td>
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<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.

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

For research use only
Version 19F11-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) 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

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. 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.
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.

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 5 min.

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>
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 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.
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 to heat FBS at 56 °C for 30 min to inactivate the alkaline phosphatase activity.

**RELATED PRODUCTS**

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<tr>
<th>Product</th>
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<tr>
<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™ hTLR2</td>
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<td>THP1-Blue™ NF-κB Cells</td>
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<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](https://www.invivogen.com/reporter-cells)
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>
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<th>Product</th>
<th>Catalog Code</th>
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<td>pSelect-zeo-Lucia™ (expression plasmid)</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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