HCT116-Dual™ Cells

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

Catalog # hctd-nfis

For research use only

Version # 16I20-MM

PRODUC T INF O RM AT IO N

Contents
• 1 vial of HCT116-Dual™ Cells (3-7 x 10⁶ cells)

IMPORTANT: Cells are shipped frozen. If cells are not frozen upon arrival, contact InvivoGen immediately.

• 100 µl Blasticidin (10 mg/ml). Store blasticidin at 4 ºC or at -20 ºC. *
• 100 µl Zeocin™ (100 mg/ml). Store Zeocin™ at 4 ºC or at -20 ºC. *
• 1 ml 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 pouch of QUANTI-Blue™. Store QUANTI-Blue™ pouch at 4 ºC for 6 months. Reconstituted QUANTI-Blue™ medium is stable for 2 weeks at 4 ºC. Protect QUANTI-Blue™ from light.
• 1 pouch of QUANTI-Luc™. Store QUANTI-Luc™ pouch at -20 ºC. Reconstituted QUANTI-Luc™ medium is stable for 12 months. Reconstituted QUANTI-Luc™ medium is stable for 1 week at 4 ºC or for 1 month at -20 ºC. Protect QUANTI-Luc™ from light.

Handling Cells Upon Arrival

We strongly recommend that you propagate the cells, using the provided procedure, as soon as possible. This will ensure the best cell viability and assay performance. Frozen cells may be placed in liquid nitrogen until you are ready to thaw and propagate them, however, this may reduce cell viability.

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.

HCT116-Dual™ cells should not be passaged more than 20 times to remain fully efficient. HCT116-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 has been confirmed upon stimulation of HCT116-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.
• HCT116-Dual™ cells are guaranteed mycoplasma-free.

SAFETY CONSIDERATIONS

Biosafety Level 1

CELL LINE DESCRIPTION

HCT116-Dual™ cells are adherent epithelial cells that have been derived from the human HCT116 colorectal carcinoma cell line by stable integration of two inducible reporter constructs. HCT116 cells are commonly used to study inflammatory responses in colon epithelial cells. HCT116-Dual™ cells express a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of the IL-12 p40 minimal promoter fused to five NF-κB and AP-1 binding sites. HCT116-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, HCT116-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.

HCT116-Dual™ cells express numerous pattern recognition receptors (PRRs), including the NOD-like receptors (NLRs) NOD1 and NOD2, the RIG-I-like receptor (RLR) RIG-I, and the Toll-like receptors (TLRs) TLR3 and TLR5 but not TLR2 or TLR4. 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 HCT116-Dual™ cells with the following PAMPs, Poly(I:C) (TLR3), flagellin (TLR5), Tri-DAP (NOD1), and MDP (NOD2), leads to the activation of NF-κB. 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.

HCT116-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 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.
**HANDLING PROCEDURES**

**Required Cell Culture Medium**
- Growth Medium: DMEM, 4.5 g/l glucose, 10% (v/v) fetal bovine serum (FBS), 50 U/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml Normocin™, 2 mM L-glutamine
- Freezing Medium: DMEM with 20% (v/v) FBS and 10% (v/v) DMSO
- Test Medium for use with QUANTI-Blue™: DMEM, 4.5 g/l glucose, 10% (v/v) heat-inactivated FBS (30 min at 56 °C), 50 U/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml Normocin™, 2 mM L-glutamine

*Note:* Heat-inactivated FBS is also commercially available.

**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 antibodies 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 15 ml of 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% CO₂.

**Frozen Stock Preparation**
1. Resuspend cells at a density of 5-7 x 10⁵ 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 cell grow to 100% confluency. *Note:* To ensure the best results:
   - Use HCT116-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 best results, 24 hours prior to the test we recommend to prepare a subculture at a 1:3 ratio.

**NK-xB induction**
1. Briefly 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 HCT116-Dual™ cells at 2.8 x 10⁵ 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. IL-1β at 100 ng/ml) 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™ following the instructions on the enclosed product data sheet.
8. Add 170 µl of resuspended QUANTI-Blue™ per well of a flat-bottom 96-well plate.
9. Add 30 µl of HCT116-Dual™ cells supernatant.
10. Incubate the plate at 37 °C in a CO₂ incubator for 1-8 h.
11. Determine NF-xB-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 HCT116-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. PolydA:dT/LyoVec™) 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 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**

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blasticidin</td>
<td>ant-bl-1</td>
</tr>
<tr>
<td>Poly(dA:dT)/LyoVec™</td>
<td>ttrl-patc</td>
</tr>
<tr>
<td>Poly(I:C) (HMW) / LyoVec™</td>
<td>ttrl-piclv</td>
</tr>
<tr>
<td>QUANTI-Blue™</td>
<td>rep-qb1</td>
</tr>
<tr>
<td>QUANTI-Luc™</td>
<td>rep-qlc1</td>
</tr>
<tr>
<td>Recombinant human IL-1β</td>
<td>rhil-1b</td>
</tr>
<tr>
<td>Zeocin™</td>
<td>ant-zn-1</td>
</tr>
</tbody>
</table>

**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 3-622-34-80
E-mail: info@invivogen.com

www.invivogen.com
**QUANTI-Blue™**

Medium for detection and quantification of alkaline phosphatase

Catalog # rep-qb1, rep-qb2

For research use only

Version # 16C18-MM

**PRODUCT INFORMATION**

**Contents:**

QUANTI-Blue™ is provided as packs of individually sealed pouches.

- **rep-qb1:** 5 pouches of QUANTI-Blue™
- **rep-qb2:** 10 pouches of QUANTI-Blue™

Each pouch contains everything needed to prepare 100 ml of medium for the detection and quantification of any alkaline phosphatase.

**Storage and Stability:**

- Store QUANTI-Blue™ pouches at 2-8 °C for 12 months.

**Important:** The correct storage temperature for this product is 2-8 °C (some pouches may be mislabeled).

- Reconstituted QUANTI-Blue™ medium is stable 2 weeks at 2-8 °C and 2 months at -20°C. Keep reconstituted QUANTI-Blue™ away from light.

**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™ medium changes 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 that are exploited by the use of QUANTI-Blue™.

- **Requires small samples of cell supernatants** - Samples of 10 µl are sufficient.
- **No need to process samples** - Preparation of cell lysates or heating of samples are not required.
- **Determine secreted AP activity without disturbing cells** - The same cell cultures can be repeatedly sampled for kinetic studies or further experimentation.
- **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™.
- **Wide dynamic range allows to detect low and high levels of AP**
- **Highly sensitive for quantitative measurement**
- **Extremely simple to use** - QUANTI-Blue™ consists of only one medium: 1) resuspend in water, 2) add sample, incubate at 37°C and 3) assess AP activity with the naked eye or by reading the optical density (OD) at 625-655 nm.

**METHODS**

**Preparation of QUANTI-Blue™**

- Pour the contents of one pouch of QUANTI-Blue™ in a 250 ml sterile glass bottle or flask.
- Add 100 ml of endotoxin-free water.
- Swirl gently.
- Warm QUANTI-Blue™ to 37°C for 30 min.
- Use reconstituted QUANTI-Blue™ immediately or store at 2-8 °C.

**Notes:**

- QUANTI-Blue™ may require overnight incubation at 2-8°C to ensure complete dissolution of the powder.
- **Optional:** To guarantee sterility, QUANTI-Blue™ can be filtered on a 0.2 µm membrane once complete dissolution is achieved. However, this step is **not necessary** as your cells will not be in contact with QUANTI-Blue™.

**Detection of SEAP activity from cell culture supernatants**

The following protocol refers to the use of 96-well plates. Vary your procedure accordingly depending on volumes of reagents needed based on the size of your wells. Some fetal bovine serum (FBS) may contain alkaline phosphatase that can interfere with SEAP quantification. We recommend to test the culture medium supplemented with FBS as a negative control to evaluate the presence of alkaline phosphatase in the serum.

- Aliquot 200 µl QUANTI-Blue™ per well.

**Note:** Warm QUANTI-Blue™ to 37°C before use.

- Add 20 µl supernatant of SEAP-expressing cells or cell culture medium as a negative control.

**Note:** If the negative control turns purple/blue, it means your FBS contains alkaline phosphatase. We recommend to heat the FBS used in your cell culture medium at 56 °C for 30 minutes to inactivate the alkaline phosphatase activity.

- Incubate at 37°C.
- After 15 min to 24 h incubation, assess SEAP activity with the naked eye or by reading the OD at 620-655 nm with a microplate reader.

**RELATED PRODUCTS**

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>pNiFty2-SEAP (Zeo+)</td>
<td>pmnifty2-seap</td>
</tr>
<tr>
<td>pSELECT-zeo-SEAP</td>
<td>psetz-seap</td>
</tr>
<tr>
<td>Recombinant SEAP Protein</td>
<td>rec-hseap</td>
</tr>
</tbody>
</table>

**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 3-622-34-80
E-mail: info@invivogen.com

**www.invivogen.com**
QUANTI-Luc™
A coelenterazine-based luminescence assay reagent
Catalog # rep-qlc1, rep-qlc2
For research use only
Version # 12G02-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 up to 12 months.
- Reconstituted QUANTI-Luc™ is stable 1 week at 4°C and at least 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™ 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™ reporter cell lines
(see Related Products). Lucia™ is a new secreted coelenterazine
luciferase encoded by a synthetic gene. As Lucia™ is secreted, it can
be directly measured in the cell culture medium using bioluminescent
assays.

InvivoGen provides a recombinant Lucia™ protein (see Related
Products) which is a positive control for QUANTI-Luc™. A dilution
series of the recombinant Lucia™ protein can also be used to
determine the linear range of the assay.

METHODS

Preparation of QUANTI-Luc™
Prepare the QUANTI-Luc™ assay solution as follows:
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 1 week
  at 4°C and 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
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- Prepare the QUANTI-Luc™ assay solution as described above.
2- 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.
3- Pipet 10 µl of sample per well into a 96-well white (opaque) or
  black plate, or a luminometer tube.
4- Prime the injector with the QUANTI-Luc™ assay solution and
  proceed with the measurement.

RELATED PRODUCTS

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant Lucia™ protein</td>
<td>rec-lucia</td>
</tr>
<tr>
<td>pSelect-zeo-Lucia™ (expression plasmid)</td>
<td>psetz-lucia</td>
</tr>
<tr>
<td>pNiFty3-Lucia™ (reporter plasmid)</td>
<td>pnf3-lc1</td>
</tr>
<tr>
<td>HEK-Dual™ TNF-α cells</td>
<td>hkd-tnffa</td>
</tr>
<tr>
<td>HEK-Dual™ IFN-γ cells</td>
<td>hkd-ifng</td>
</tr>
<tr>
<td>THP1-Lucia™ NF-kB cells</td>
<td>thp1-nfkb</td>
</tr>
<tr>
<td>THP1-Dual™ (NF-kB-IG) cells</td>
<td>thpd-nfis</td>
</tr>
<tr>
<td>Jurkat-Dual™ (ISG-NF-kB) cells</td>
<td>jktd-isnf</td>
</tr>
</tbody>
</table>