

HEK-Dual™ hTLR2 (NF/IL8) Cells

(NF-κB-SEAP/KI-[IL-8]Lucia)

Dual NF-κB and IL-8 reporter HEK293 cells expressing human TLR2

Catalog # hkd-htlr2ni

<http://www.invivogen.com/hek-dual-htlr2>

For research use only

Version # 17B17-MM

PRODUCT INFORMATION

Content

- 1 vial of HEK-Dual™ hTLR2 (NF/IL8) Cells (3-7 x 10⁶ cells)
- 100 µl Hygromycin B Gold (100 mg/ml). Store at 4 °C or at -20 °C.*
- 100 µl Zeocin™ (100 mg/ml). Store at 4 °C or at -20 °C.*
- 1 ml Normocin™ (50 mg/ml). Normocin™ is a formulation of 3 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™ (SEAP detection medium)

Store QUANTI-Blue™ pouch at 4 °C for 6 months. Reconstituted QUANTI-Blue™ medium is stable for 2 weeks at 4 °C. Protect from light.

- 1 pouch of QUANTI-Luc™ (Lucia luciferase detection medium)

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.

Handling Cells Upon Receipt

Cells must be thawed **immediately** upon receipt and grown according to handling procedures (see 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.

Cell Line Stability

Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Cells will undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages. To ensure maximum efficiency, do not passage HEK-Dual™ hTLR2 (NF/IL8) Cells more than 20 times. These cells should be maintained in growth medium supplemented with selective antibiotics, Hygromycin B Gold (100 µg/ml) and Zeocin™ (50 µg/ml).

Quality Control

- The biallelic replacement of the human interleukin-8 (IL-8) open reading frame (ORF) with the Lucia luciferase ORF has been verified by PCR and sequencing. Furthermore, the inability to produce IL-8 has been confirmed by ELISA.
- TLR3, TLR5 and TNF receptor (TNFR) knockout has been verified by functional assays and DNA sequencing to confirm frameshift deletion.
- The expression of human TLR2 (hTLR2) and the CD14 co-receptor has been confirmed by flow cytometry.
- The response to various TLR agonists has been tested. As expected, only TLR2 agonists induced the production of the reporter proteins.
- The cell line stability for 20 passages following thawing has been verified.
- The cell line is guaranteed mycoplasma-free.

BACKGROUND

HEK-Dual™ TLR (NF/IL8) cells are a family of Toll-like receptor (TLR)-reporter cells designed for the study of the inflammatory response induced by the stimulation of a given TLR by monitoring the activation of the transcription factors NF-κB and AP-1 and/or the expression of interleukin-8 (IL-8). IL-8 is a chemokine produced in response to TLR agonists in an NF-κB and AP-1 dependent-manner^{1,2}. HEK-Dual™ TLR (NF/IL8) cells were generated from the human embryonic kidney 293 (HEK293)-derived cell line, HEK-Dual™ Null (NF/IL8), by stable transfection of a TLR gene. This parental cell line, that features a triple knockout of TLR3, TLR5 and TNFR (all of which are endogenously expressed in HEK293 cells), stably expresses an NF-κB/AP-1 inducible secreted embryonic alkaline phosphatase (SEAP) reporter construct. It also expresses Lucia luciferase, a secreted luciferase, placed under the control of the endogenous IL-8 promoter; the coding sequence of IL-8 was replaced by the Lucia luciferase ORF using knockin technology. Thus, TLR stimulation can be assessed in HEK-Dual™ TLR (NF/IL8) cells by monitoring NF-κB/AP-1-induced SEAP production and/or IL-8-dependent expression of Lucia luciferase.

The two reporter proteins, SEAP and Lucia Luciferase, can be readily measured in the supernatant by using QUANTI-Blue™ and QUANTI-Luc™, respectively.

1. Ohta K. et al., 2014. Toll-like receptor (TLR) expression and TLR-mediated interleukin-8 production by human submandibular gland epithelial cells. *Mol Med Rep.* 10(5):2377-82. **2. Roebuck KA. et al., 1999.** Regulation of interleukin-8 gene expression. *J Interferon Cytokine Res.* 19(5):429-38.

CELL LINE DESCRIPTION

HEK-Dual™ hTLR2 (NF/IL8) cells were generated from HEK-Dual™ Null cells by stable transfection of the human TLR2 (hTLR2) and CD14 genes. Due to the knockout of TLR3 and TLR5, these cells enable the study of hTLR2 signaling without interference from other TLRs. They respond to very low concentrations of TLR2 agonists such as the synthetic lipopeptide Pam3CSK4. They do not respond to other TLR agonists or to the cytokine TNF-α (see validation sheet).

Note: HEK-Dual™ hTLR2 (NF/IL8) and their parental cell line endogenously express NOD1.

HEK-Dual™ hTLR2 (NF/IL8) cells are resistant to hygromycin B, Zeocin™ and blasticidin. They should be maintained in growth medium (see next page) supplemented with hygromycin B and Zeocin™.

TECHNICAL SUPPORT

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SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Dual™ hTLR2 (NF/IL8) cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) and thus may require Biosafety Level 2. The biosafety level varies by country. In the United States, HEK293 cell lines are designated Biosafety Level 2 according to the Center for Disease Control and Prevention (CDC). In Germany, HEK293 cell lines are designated Biosafety Level 1 according to the Central Committee of Biological Safety, Zentrale Kommission für die Biologische Sicherheit (ZKBS). Please check with your country's regulatory authority regarding the use of these cells.

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: 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 Antibiotics

Hygromycin B Gold 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% (v/v) ethanol.

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

3. Transfer cells into 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 25 cm² tissue culture flask containing 5 ml of growth medium without selective antibiotics.
7. Place the flask containing cells at 37 °C in 5% CO₂.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10⁶ cells/ml in freezing medium freshly prepared with cold growth medium.

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

2. Dispense 1 ml of the cell suspension into cryogenic vials.
3. Place vials in a freezing container (Nalgene) 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 (after at least one passage), subculture the cells in growth medium supplemented with 100 µg/ml of Hygromycin B Gold and 50 µg/ml of Zeocin™.
2. Renew growth medium twice a week. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

Induction of HEK-Dual™ hTLR2 (NF/IL8) Cells

Day 1:

1. Add 20 µl of each sample per well of a flat-bottom 96-well plate.
2. Add 20 µl of a positive control such as FSL-1 at 100 pg/ml in one well.
3. Add 20 µl of a negative control such as sterile, endotoxin-free water in another well.
4. Prepare a cell suspension of HEK-Dual™ hTLR2 (NF/IL8) Cells at ~280,000 cells per ml in test medium (containing 10% v/v heat-inactivated FBS).

Note: Some FBS may contain alkaline phosphatases that can interfere with SEAP quantification. We recommend to use heat-inactivated FBS to inactivate these enzymes which are thermosensitive.

5. Add 180 µl of cell suspension (~50,000 cells) per well.
6. Incubate the plate at 37 °C in a CO₂ incubator for 20-24 h.

Detection of the NF-κB response using QUANTI-Blue™

Day 2:

1. Prepare QUANTI-Blue™ following the instructions on the enclosed technical data sheet (TDS).
2. Add 180 µl of resuspended QUANTI-Blue™ per well of a flat-bottom 96-well plate.
3. Add 20 µl of induced HEK-Dual™ hTLR2 (NF/IL8) cell culture supernatant.
4. Incubate the plate at 37 °C incubator for 1-3 h.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

Detection of the IL-8 response using QUANTI-Luc™

Below is a protocol for end-point readings using a luminometer, this protocol can be adapted for use with kinetic measurements.

Day 2:

1. Prepare QUANTI-Luc™ following the instructions on the enclosed TDS.
2. Pipet 10 µl of HEK-Dual™ hTLR2 (NF/IL8) cell culture supernatant per well in a 96-well white (opaque) or black plate, or a luminometer tube.
3. Add 50 µl of QUANTI-Luc™ per well.
4. Proceed **immediately** with the measurement.

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

RELATED PRODUCTS

Product	Catalog Code
HEK-Dual™ Null Cells	hkd-null
HKLM (Heat Killed <i>L. monocytogenes</i> ; TLR2 ligand)	tlrl-hklm
Hygromycin B Gold	ant-hg-1
FSL-1 (Synthetic diacylated lipoprotein; TLR2/6 ligand)	tlrl-fsl
Pam3CSK4 (Synthetic triacylated lipoprotein; TLR1/2 ligand)	tlrl-pms
QUANTI-Blue™	rep-qb1
QUANTI-Luc™	rep-qlc1
Zeocin™	ant-zn-1

TECHNICAL SUPPORT

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QUANTI-Blue™

Medium for detection and quantification of alkaline phosphatase

Catalog # rep-qb1, rep-qb2
<http://www.invivogen.com/quant-blue>

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**
No need to perform multiple sample dilutions.
- **Highly sensitive for quantitative measurement**
Higher saturation threshold than with pNPP resulting in more significant differences between non or low AP expression and high AP expression.
- **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.

	96-well plate	24-well plate	12-well plate
QUANTI-Blue™	200 µl	500 µl	1 ml
Supernatant	20/5 µl	50/10/5 µl	100/25/10 µl

RELATED PRODUCTS

Product	Catalog Code
pNiFty2-SEAP (Zeo®)	pnifty2-seap
pSELECT-zeo-SEAP	psetz-seap
Recombinant SEAP Protein	rec-hseap

TECHNICAL SUPPORT

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QUANTI-Luc™

A coelenterazine-based luminescence assay reagent

Catalog # rep-qlc1, rep-qlc2
<http://www.invivogen.com/quantiluc>

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 quantitatively 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

Product	Catalog Code
Recombinant Lucia™ protein	rec-lucia
pSelect-zeo-Lucia™ (expression plasmid)	psetz-lucia
pNiFty3-Lucia™ (reporter plasmid)	pnf3-lc1
HEK-Dual™ TNF-α cells	hkd-tnfa
HEK-Dual™ IFN-γ cells	hkd-ifng
THP1-Lucia™ NF-κB cells	thp1-nfkb
THP1-Dual™ (NF-κB-ISG) cells	thpd-nfis
Jurkat-Dual™ (ISG-NF-κB) cells	jktd-isnf

TECHNICAL SUPPORT

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