HEK-Dual™ RNA-Null Cells

RNA sensor deficient - HEK293 reporter cells

Catalog code: hkd-rna-null

https://www.invivogen.com/hek-dual-rna-sensor-null

For research use only

Version 25A21-AK

PRODUCT INFORMATION

Content

- 3-7 x 10^6 of HEK-Dual™ RNA-Null 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), 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 tube of QUANTI-Luc™ 4 Reagent**, a lucia luciferase detection reagent (sufficient to prepare 25 ml). Store at -20°C. Avoid repeated freeze-thaw cycles. *Note: This product is photosensitive and should be protected 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 Frozen Cells Upon Arrival

Cells are shipped in dry ice, and upon receipt should immediately be thawed for culture or stored below -130°C, preferably in liquid nitrogen vapor, for long-term storage.

IMPORTANT: Do not store cell vials at -80°C as this will decrease cell viability and performance. Contact technical support if the cells are not frozen or in dry ice upon arrival.

To insure the highest level of viability and best assay performance, we strongly recommend that you thaw the cells and initiate the culture as soon as possible upon receipt (as described on the next page).

Warranties

- InvivoGen's cells are provided 'AS IS' and their viability is guaranteed upon shipment from our facilities for a period of 30 days, provided that the customer has properly stored and handled the product.
- Our cell lines are guaranteed free of mycoplasma contamination.
- The stability of our cell lines is guaranteed for 20 passages.

Quality Control

- The deletion of the human *RIG-I*, *MDA5*, and *TLR3* genes have been verified by PCR, Western Blot, and functional assays.
- The stability for 20 passages following thawing has been verified.
- The cell line is guaranteed mycoplasma-free.

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 outlicensing@invivogen.com.

PRODUCT DESCRIPTION

HEK-Dual™ RNA-Null cells are designed to assess the distinct role of double-stranded (ds) RNA sensors. They are derived from the human embryonic kidney 293 (HEK293)-Dual™ cell line harboring the stable integration of two inducible reporter genes for SEAP (secreted embryonic alkaline phosphatase) and Lucia luciferase. As a result, these cells allow the simultaneous study of the NF-κB pathway, by monitoring the activity of SEAP, and the IRF (interferon regulatory factor) pathway, by assessing the activity of a secreted luciferase.

HEK-Dual™ RNA-Null cells lack three important dsRNA sensors MDA5 (Melanoma differentiation-associated gene 5), RIG-I (Retinoic acid-inducible protein 1), and TLR3 (Toll-like receptor 3). Due to the absence of these sensors, HEK-Dual™ RNA-Null cells do not respond to any synthetic dsRNA analog compared to their parental cell line HEK-Dual™. These cells are selectable with Blasticidin and Zeocin®.

BACKGROUND

The antiviral response is initiated through the recognition of viral products, such as double-stranded (ds) RNA, by three major pathogen recognition receptors (PRRs)[1,2]: the RIG-I-like receptors (RLRs) MDA5 and RIG-I, as well as the Toll-like receptor 3 (TLR3). Upon recognition of viral or synthetic dsRNA, these major PRRs mediate cell survival, the transcriptional induction of proinflammatory cytokines, chemokines, and type I interferons (IFNs), thereby inducing hundreds of interferon-stimulated genes (ISGs). These products have antiviral, immunomodulatory, cell growth regulatory, and metabolic regulatory actions (e.g. apoptosis) that create an antiviral state. If successful, this response potently restricts virus replication and cell-to-cell spread of infection[3].

1. Qiao, H, *et al.*, 2021. Cell fate determined by the activation balance between PKR and SPHK1. Cell Death Differ 28, 401–418. **2. Kawai T. *et al.*, 2005.** IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. Nat Immunol. 6(10):981-988. **3. Kell AM, Gale M Jr., 2015.** RIG-I in RNA virus recognition. Virology.;479-480:110-21.

SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Dual™ RNA-Null 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.

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FAQ Any questions about our cell lines? Visit our FAQ page.

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% (v/v) heat-inactivated FBS, 100 µg/ml Normocin®, Pen-Strep (100 U/ml-100 µg/ml)
- **Freezing Medium:** DMEM, 20% (v/v) FBS, 10% (v/v) DMSO
- **Test Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% (v/v) heat-inactivated FBS, Pen-Strep (100 U/ml-100 µg/ml) **without Blasticidin, Normocin®, and Zeocin®.**

Required Selective Antibiotics

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% (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 thawing medium (should contain 20% FBS). **Do not add selective antibiotics until the cells have been passaged twice.**
4. Centrifuge vial at 300 x g (RCF) 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 cm^2 tissue culture flask containing 8 ml of thawing medium without selective antibiotics.
7. Place the flask containing cells at 37 °C in 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 3-5 x 10^6 cells/ml in freshly prepared freezing medium with cold DMEM.

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. HEK-Dual™ RNA-Null cells grow as adherent cells.

Detach the cells using **undiluted** trypsin for 2-3 min at room temperature (RT).

Note: Prolonged action of trypsin or incubation at 37°C may alter the cell surface expression of receptors.

2. After cells have recovered (after at least one passage), subculture the cells in growth medium supplemented with 10 µg/ml of Blasticidin and 100 µg/ml of Zeocin®.
3. Examine the cell culture **every weekday**. Renew growth media regularly and add extra volume over the weekend.
4. Cells should be passaged when a 60-80% confluency is reached. Do not let the cells grow to 100% confluency. When passing the cells, start the cell culture at about 20% confluency.

Note: This cell line grows slower than its parental cell line HEK-Dual™.

REPORTER ASSAY PROTOCOL

Below is a protocol using HEK-Dual™ RNA-Null cells as a control to monitor the TLR3-dependent NF-κB and IRF responses in HEK-Dual™ RNA-hTLR3 cells. To avoid high background signal, change the culture medium to test medium the day before the assay. Additionally, it is recommended to perform the assay with test medium that does not contain Blasticidin, Normocin®, nor Zeocin®.

Induction of HEK-Dual™-derived cells

1. Add 20 µl of sample (e.g. Poly(I:C) HMW at 10 µg/ml) per well of a flat-bottom 96-well plate.
2. Include a NF-κB positive control (e.g. recombinant hTNF-α at 10 ng/ml), an IRF positive control (e.g. recombinant hIFN-β at 30 U/ml), and a negative control (e.g. endotoxin free water).
3. Remove old media and wash HEK-Dual™ RNA-Null cells and HEK-Dual™ RNA-hTLR3 cells with 1XPBS.
4. Detach cells using undiluted trypsin for 2-3 min at RT.

Note: The response of HEK-Dual™-derived cells can be altered by the prolonged action of trypsin. Do not incubate with trypsin at 37°C and for no longer than 2-3 min. Using PBS to detach the cells might increase the background activity of NF-κB and/or IRF.

5. Add freshly prepared, pre-warmed test medium to stop the reaction, count your cells, and centrifuge at 300 x g (RCF) for 5 minutes.
6. Resuspend cells in test medium to prepare a cell suspension at ~280,000 cells/ml.
7. Add 180 µl of cell suspension (~50.000 cells) per well.
8. Incubate overnight at 37 °C in 5% CO_2.

Detection of the NF-κB response

1. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed product data sheet.
2. Add 20 µl of induced cell culture supernatant.
3. Add 180 µl of resuspended QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
4. Incubate the plate at 37 °C for 30 min to 1 hour.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

Detection of the IRF response

Below is a protocol for end-point readings using a luminometer with an injector. This protocol can be adapted for use with a luminometer with or without an injector for kinetic measurements.

1. Prepare QUANTI-Luc™ 4 Reagent working solution following the instructions on the data sheet.
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 - 20 µl of cell culture supernatant per well in a 96-well white (opaque) or black plate, or a luminometer tube.
4. Prime the injector with QUANTI-Luc™ 4 Reagent working solution
5. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Cat. Code
HEK-Dual™ cells	hkd-nfis
HEK-Dual™ RNA-hMDA5 cells	hkd-rna-mda5
HEK-Dual™ RNA-hRIG-I cells	hkd-rna-rigi
HEK-Dual™ RNA-hTLR3 cells	hkd-rna-tlr3
QUANTI-Blue™ Solution	rep-qbs
QUANTI-Luc™ 4 Lucia/Gaussia	rep-qlc4lg1
Poly(I:C) HMW VacciGrade™	vac-pic
Blasticidin	ant-bl-05
Normocin®	ant-nr-1
Zeocin®	ant-zn-1

TECHNICAL SUPPORT
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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

For research use only

Version 23C09-MM

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**.

Note: During storage, a precipitate may form in the 20 ml bottle of QB reagent and QB buffer. If this occurs, heat the product at 37°C for 30 seconds and vortex until the precipitate disappears. The formation of a precipitate does not affect the activity of the product.

- Reconstituted QUANTI-Blue™ Solution is stable for 2 weeks at 2-8 °C and for 2 months at -20°C. Protect from light.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.

- Physicochemical characterization (pH, appearance).
- 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 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.

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

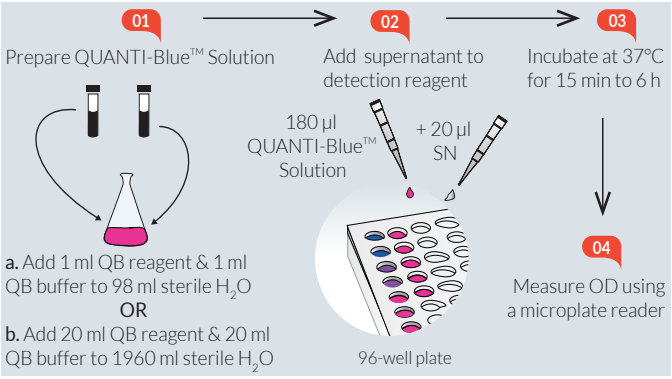

Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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:
   a. **1 ml** of QB reagent and **1 ml** of QB buffer to **98 ml** of sterile water.
   b. **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.

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.

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

	96-well plate	24-well plate	12-well plate
QUANTI-Blue™	180 µl	450 µl	900 µl
Supernatant	20 µl	50 µl	100 µl

TECHNICAL SUPPORT
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B. High Throughput Screening (HTS) procedure

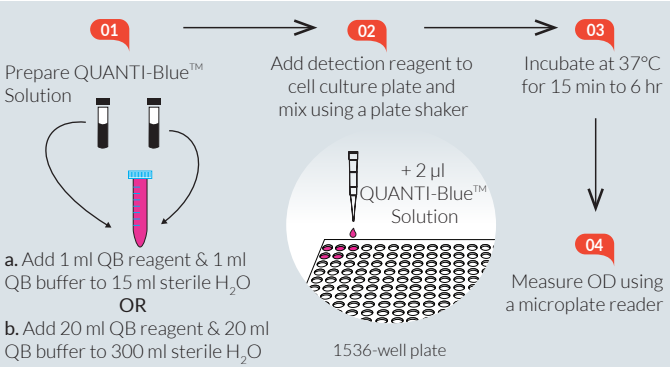

Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

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** of QB reagent and **1 ml** of QB buffer to **15 ml** of sterile water in a sterile 50 ml screw cap tube.
   b. **20 ml** of QB reagent and **20 ml** of QB buffer to **300 ml** of sterile water in a sterile glass bottle or flask.
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

Product	Catalog Code
pNiFty2-SEAP (Zeo®)	pnifty2-seap
pSELECT-zeo-SEAP	psetz-seap
HEK-Blue™ Detection	hb-det2
Recombinant SEAP Protein	rec-hseap
Reporter cells	
HEK-Blue™ hTLR2	hkb-htlr2
HEK-Blue™ hTLR4	hkb-htlr4
RAW-Blue™ Cells	raw-sp
THP1-Blue™ NF-κB Cells	thp-nfkb
THP1-Blue™ ISG Cells	thp-isg

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

QUANTI-Luc™ 4 Reagent

A coelenterazine-based luminescence assay reagent

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

For research use only

Version 24G30-MM

PRODUCT INFORMATION

Contents

- 1 tube of **QUANTI-Luc™ 4 Reagent (20X)**

  One tube of QUANTI-Luc™ 4 Reagent is sufficient for 5 x 96-well plates (25 ml standard Flash/end-point detection).

Note: This sample cannot be sold separately from the QUANTI-Luc™ 4 Lucia/Gaussia or Renilla kits.

Find more information at https://www.invivogen.com/quanti-luc.

Storage and Stability

– Store QUANTI-Luc™ 4 Reagent at -20°C for up to 12 months.
– After preparation, the working solution is stable for 48 hours 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.

Quality Control

Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.
– Physicochemical characterization (pH, appearance).
– Functional assays using recombinant Lucia® protein or reporter cells.

DESCRIPTION

QUANTI-Luc™ 4 Reagent is one component of the QUANTI-Luc™ 4 Lucia/Gaussia and QUANTI-Luc™ 4 Renilla kits. It contains the coelenterazine substrate for the detection of secreted Lucia® or Gaussia activity in live-cell supernatants, and of intracellular Renilla after cell lysis. The light signal produced correlates to the amount of luciferase protein expressed. It is quantified using a luminometer and expressed as relative light units (RLUs).

Note: Lucia® is a registered trademark of InvivoGen.

METHODS

Preparation of QUANTI-Luc™ 4 Reagent working solution (1X):

1. Dilute the total volume of the 20X tube (1.25 ml) of Reagent into 23.75 ml of sterile water to obtain 25 ml of working solution.
2. Vortex **very briefly** (a few seconds).
3. Use the working solution immediately or store until required for use. QUANTI-Luc™ 4 Reagent working solution can be stored for 48 hours at 4°C or 1 month at -20°C.

Flash detection of Lucia® luciferase activity in 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 QUANTI-Luc™ 4 Reagent 1X 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™ 4 Reagent 1X 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

Product	Cat. Code
QUANTI-Luc™ 4 Lucia/Gaussia Kit comprising QUANTI-Luc™ 4 Reagent & Stabilizer	rep-qlc4lg1
QUANTI-Luc™ 4 Renilla Kit comprising QUANTI-Luc™ 4 Reagent & Lysis buffer	rep-qlc4r1

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