

HEK-Dual™ TNF-α Cells

TNF-α Sensor Cells

Catalog code: hkd-tnfa

<https://www.invivogen.com/hek-dual-tnf-a>

For research use only

Version 19K24-MM

PRODUCT INFORMATION

Contents

- 1 vial of HEK-Dual™ TNF-α cells (3-7 x 10⁶ cells)
 - 1 ml of Normocin™ (50 mg/ml), a formulation of three antibiotics active against mycoplasmas, bacteria, and fungi. Store at -20°C.*
 - 1 ml of Zeocin™ (100 mg/ml). Store at 4°C or 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). QB reagent and QB buffer are stable for 1 year 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 and for 1 month at -20°C. Protect QUANTI-Luc™ from light.

Note: Data sheets for all components are available on our website.

Handling 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.

Cell Line Stability

Cells will undergo genotypic changes over time that will result in reduced responsiveness 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.

Quality Control

- SEAP reporter activity in response to TNF-α and various cytokines has been validated using functional assays.
- The stability for 20 passages, following thawing, has been verified.
- These cells are guaranteed mycoplasma-free.

USE RESTRICTIONS

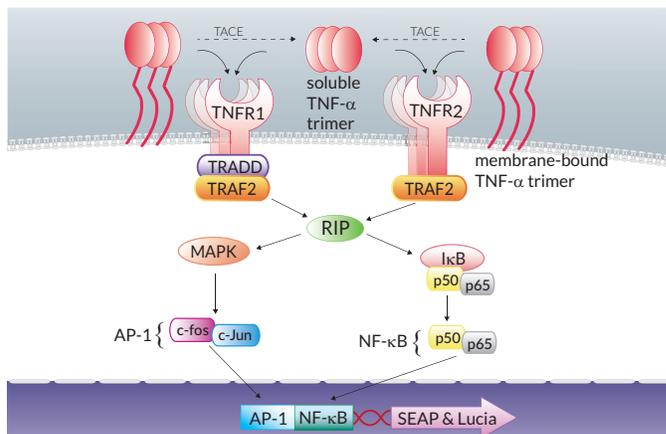
These cells are distributed for research purposes only.

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BACKGROUND

Tumor necrosis factor-alpha (TNF-α) is a pleiotropic cytokine involved in necrotic and apoptotic cell death, cellular differentiation, inflammation, and regulation of immune cell activity¹. TNF-α is mainly produced by activated monocytes, macrophages, and T cells. It is first synthesized as a membrane-bound molecule (memTNF-α) that is cleaved by tumor necrosis factor-alpha converting enzyme (TACE) resulting in the soluble trimeric form (solTNF-α)². Both forms bind homotrimeric transmembrane receptors, TNFR1 or TNFR2, triggering signaling pathways that involve TRADD, TRAF2 and RIP, and leads to the activation of NF-κB and MAPK pathways.

1. Steeland S. et al., 2018. A new venue of TNF targeting. Int. J. Mol. Sci. 19:1442.
2. Brenner D. et al., 2015. Regulation of tumour necrosis factor signalling: live or let die. Nat Rev Immunol. 15(6):362-74.



CELL LINE DESCRIPTION

HEK-Dual™ TNF-α cells were designed for the detection of bioactive human TNF-α by monitoring the activation of the AP-1/NF-κB pathway. These cells derive from the human embryonic kidney 293 cell line by stable co-transfection of two NF-κB-inducible reporter constructs; the SEAP (secreted embryonic alkaline phosphatase) gene and the Lucia luciferase reporter gene. Both reporter genes are under the control of the same NF-κB-inducible promoter consisting of 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. Both reporter proteins are readily measurable in the cell culture supernatant when using QUANTI-Blue™ Solution, a SEAP detection reagent, and QUANTI-Luc™, a Lucia™ detection reagent. Of note, HEK-Dual™ TNF-α cells do not respond to IL-1β due to the stable knockout of the MyD88 gene. HEK-Dual™ TNF-α cells are resistant to the selectable marker Zeocin™.

Detection range for hTNF-α using QUANTI-Luc™: 0.5 ng - 1 µg/ml

Detection range for hTNF-α using QUANTI-Blue™: 1 ng - 1 µg/ml

TECHNICAL SUPPORT

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SAFETY CONSIDERATIONS

Biosafety Level 2

HEK-Dual™ TNF- α cells were derived from HEK293 cells (transformed with adenovirus 5 DNA) that require **Biosafety level 2** according to the American Center for Disease Control and Prevention (CDC) guidelines. The biosafety level may vary depending on the country. For example, 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, 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum (FBS; 30 min at 56 °C), 100 μ g/ml Normocin™, Pen-Strep (100 U/ml-100 μ g/ml)
- **Freezing Medium:** DMEM, 4.5 g/l glucose, 20% FBS, 10% DMSO
- **Test Medium:** DMEM 4.5 g/l glucose, 2 mM L-glutamine, 10% heat-inactivated FBS, Pen-Strep (100 U/ml-100 μ g/ml) **without Normocin™ and Zeocin™**

Required Selection Antibiotics

- 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 to a larger tube containing 15 ml of pre-warmed growth medium. **Do not add Zeocin™ until the cells have been passaged twice.**
4. Centrifuge 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 selection antibiotics.
6. Transfer the cells 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 10⁶ cells/ml in freezing medium freshly prepared with cold DMEM.
Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.
2. Prepare 1 ml aliquots of cells in 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. Maintain and subculture the cells in growth medium supplemented with 100 μ g/ml of Zeocin™.
2. Renew the growth medium twice a week.
3. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

Cell Handling Recommendations

To ensure the best results:

- Use HEK-Dual™ TNF- α cells with less than 20 passages.

Induction of HEK-Dual™ TNF- α Cells

Day 1:

1. Add 20 μ l of each sample per well of a flat-bottom 96-well plate.
2. Add 20 μ l of TNF- α at 10 ng/ml stock solution (positive control) in one well.
3. Add 20 μ l of IL-1 β at 10 ng/ml (negative control) in one well.
4. Prepare a cell suspension of HEK-Dual™ TNF- α 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 μ l of cell suspension (~50,000 cells) per well.
6. Incubate the plate at 37 °C in a CO₂ incubator for 20-24 h.

The induction of HEK-Dual™ TNF- α cells can be detected using either the SEAP reporter with QUANTI-Blue™ or the Lucia luciferase reporter with QUANTI-Luc™.

TNF- α Detection using QUANTI-Blue™ Solution

Day 2:

1. Prepare QUANTI-Blue™ Solution following the instructions on the pouch.
2. Add 180 μ l of resuspended QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
3. Add 20 μ l of induced HEK-Dual™ TNF- α 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.

TNF- α Detection using QUANTI-Luc™

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.

Day 2:

1. Prepare QUANTI-Luc™ following the instructions on the pouch.
2. Set the luminometer with the following parameters: 50 μ l of injection, end-point measurement with a 4 second start time and 0.1 second reading time.
3. Pipet 10 μ l of HEK-Dual™ TNF- α cell culture supernatant per well in 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	Description	Cat. Code
Anti-hTNF- α -IgG1	Neutralizing antibody	htnfa-mab1
Normocin™	Antimicrobial reagent	ant-nr-1
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
Recombinant human IL-1 β	Recombinant cytokine	rcyc-hil1b
Recombinant human TNF- α	Recombinant cytokine	rcyc-htnfa
Zeocin™	Selective antibiotic	ant-zn-1

TECHNICAL SUPPORT

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QUANTI-Luc™

A coelenterazine-based luminescence assay reagent

Catalog code: rep-qlc1, rep-qlc2

<https://www.invivogen.com/quantiluc>

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 quantitatively 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

Product	Catalog Code
QUANTI-Luc™ Gold (For standard and HTS assays)	rep-qlcg1
pSelect-zeo-Lucia™ (expression plasmid)	psetz-lucia
Recombinant Lucia luciferase protein	rec-lucia
Reporter Cells	
THP1-Dual™ (IRF-Lucia/NF-κB-SEAP) Cells	thpd-nfis
THP1-Lucia™ NF-κB Cells	thp1-nfkb

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

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

<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

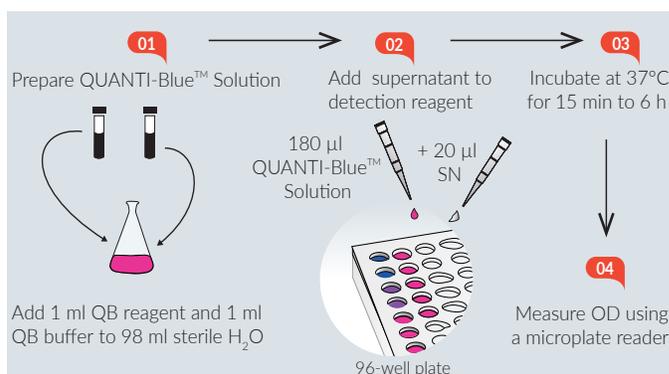


Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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. 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. 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 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 to heat 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

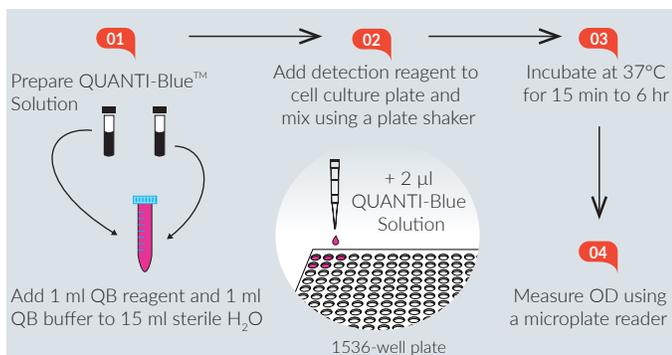


Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

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 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

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>

TECHNICAL SUPPORT

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