THP1-Dual™ KO-MyD Cells

MyD88 knockout NF-κB-SEAP and IRF-Lucia luciferase reporter monocytes

Catalog code: thpd-komyd

https://www.invivogen.com/thp1-dual-komyd

For research use only

Version 23E26-MM

PRODUCT INFORMATION

Contents

- **3-7 x 10^6 THP1-Dual™ KO-MyD 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 -20 °C.*
- **1 ml of Zeocin®** (100 mg/ml). Store at 4°C or -20 °C.*
- **1 ml of Normocin™** (50 mg/ml), 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 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). 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.

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

Handling Frozen 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.

IMPORTANT: For cells that arrive in a shipping flask please refer to the enclosed 'cell recovery procedure'.

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 genetically engineered cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages.

Quality Control

- Biallelic *MyD88* gene knockout (KO) has been verified by PCR, DNA sequencing, Western Blot, and 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.

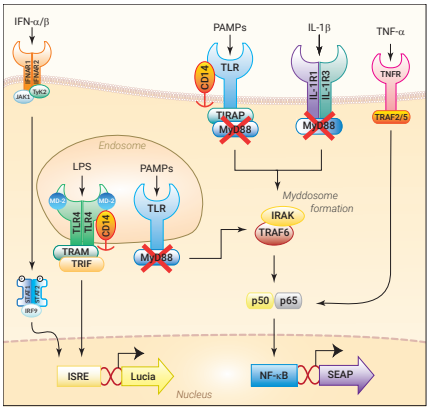
This product is covered by a Limited Use License. By use of this product, the buyer agrees with the terms and conditions of all applicable Limited Use Label Licenses. For non-research use, such as screening, quality control or clinical development, contact info@invivogen.com.

PRODUCT DESCRIPTION

THP1-Dual™ KO-MyD cells are designed to use in combination with their parental cell line THP1-Dual™ to monitor the MyD88-dependent NF-κB pathway.

THP1-Dual™ KO-MyD cells were generated from the human THP1-Dual™ cell line through the stable knockout (KO) of the *MyD88* gene. They also feature two reporter genes allowing the simultaneous study of the IRF pathway, by monitoring the activity of an inducible secreted Lucia luciferase and the NF-κB pathway by monitoring the activity of an inducible SEAP (secreted embryonic alkaline phosphatase). Lucia luciferase and SEAP activities are readily assessable in the supernatant using QUANTI-Luc™ 4 Lucia/Gaussia and QUANTI-Blue™ Solution, respectively.

THP1-Dual™ KO-MyD cells are unable to respond to the activation of receptors whose signaling is dependent on MyD88, such as TLR2, TLR4, TLR5, TLR8 and IL-1Rs, when compared to their parental cell line THP1-Dual™. However, they remain responsive to MyD88-independent receptors. The activation of the IRF pathway is unaffected by the KO. Moreover, this KO cell line can be used together with InvivoGen's THP1-Dual™ KO-TRIF to compare MyD88 and TRIF-dependent responses. These cells are selectable with Blasticidin and Zeocin®.



BACKGROUND

The myeloid differentiation primary response protein 88 (MyD88) is a ubiquitously expressed cytoplasmic adaptor protein that plays a central role in the toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) signaling pathways. Stimulation of TLRs or IL1R leads to the recruitment of cytosolic MyD88 and the formation of the so-called Myddosome. This oligomeric protein complex comprises of MyD88 and IL-1R associated kinases (IRAKs). Upon phospholylation and interaction with TRAF6, NF-κB is released, migrates to the nucleus and induces gene expression. Based on its biological and complex role, MyD88 has been in the spotlight as a therapeutic target for different inflammatory disorders [1].

1. Chen L, *et al.*, 2020. Myeloid Differentiation Primary Response Protein 88 (MyD88): The Central Hub of TLR/IL-1R Signaling. J Med Chem. ;63(22):13316-13329.

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 Asia: +852 3622-3480
E-mail: info@invivogen.com

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

InvivoGen
www.invivogen.com

SAFETY CONSIDERATIONS

Biosafety Level: 1

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% (v/v) heat-inactivated fetal bovine serum (FBS; 30 min at 56°C), 100 µg/ml Normocin™, Pen-Strep (100 U/ml-100 µg/ml)

Initial culture of all THP-1 derived cells must be performed in growth medium containing 20% heat-inactivated FBS.

Note: The use of Normocin™ together with Pen-Strep is required to keep the cells free of microbial contaminants. Contamination of this cell line may activate TLRs resulting in differentiation of the monocytes and activation of the reporter gene.

- **Test Medium:** RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated fetal bovine serum, Pen-Strep (100 U/ml-100 µg/ml)
- **Freezing Medium:** 95% FBS and 5% DMSO

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% ethanol.

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

3. Transfer cells in a vial containing 15 ml of pre-warmed growth medium (with 20% heat-inactivated FBS). **Do not add selective antibiotics until the cells have been passaged twice.**
4. Centrifuge vial at 150 x g (RCF) for 10 minutes.
5. Remove supernatant containing the cryoprotective agent and resuspend cells with 1 ml of growth medium (with 20% heat-inactivated FBS).
6. Transfer the vial contents to a 25 cm^2 tissue culture flask containing 5 ml of growth medium (with 20% heat-inactivated FBS).
7. Place the culture at 37°C, 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10^6 cells/ml in freshly prepared freezing medium freshly prepared with cold FBS.

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 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 (following at least two passages), maintain and subculture the cells in growth medium. To maintain selection pressure, add 10 µg/ml Blasticidin and 100 µg/ml Zeocin® to the growth medium every other passage.
2. Pass the cells every 3 days by inoculating 5 x 10^5 cells/ml. Do not allow the cell concentration to exceed 2 x 10^6 cells/ml.

Note: The average doubling time for the THP1-Dual™ KO-MyD cells is 36 - 48 hours using the conditions described above.

Optional: PMA induced THP1 Differentiation

Following Phorbol 12-myristate 13-acetate (PMA) treatment, **THP1-Dual™ cells** are more sensitive to IRF-inducers, such as LPS (i.e. TLR4-dependent TRIF activation).

Note: PMA treatment may activate NF-κB, and thus, lead to higher background readings with QUANTI-Blue™ Solution.

1. Add 180 µl of THP1-Dual™ KO-MyD cell suspension per well of a 96-well plate (~ 200,000 cells/well).
2. Add 20 µl of PMA (final concentration 10 ng/ml) to the cells for 3 hours at 37°C in 5% CO_2.
3. Wash cells gently with pre-warmed PBS and add 200 µl of pre-warmed growth medium.
4. Incubate for 3 days at 37°C in 5% CO_2.
5. Wash cells with pre-warmed PBS and add 180 µl of growth medium.
6. Proceed with the reporter assay as described below.

REPORTER ASSAY PROTOCOL

Below is a protocol using THP1-Dual™ KO-MyD cells together with THP1-Dual™ cells to monitor the IRF response and the MyD88-dependent NF-κB reponse. It is recommended to perform the assay with test medium which does not contain Normocin™, Zeocin® nor Blasticidin.

Cell preparation

1. Centrifuge at 150 x g (RCF) for 10 mins or 300 x g (RCF) for 5 mins.
2. Remove supernatant
3. Prepare a cell suspension of THP1-Dual™ cells and THP1-Dual™ KO-MyD cells in freshly prepared, pre-warmed test medium at 5 x 10^5 cells/ml.

Cell stimulation

1. Add 20 µl of ligands (e.g. 1 µg/ml LPS-EB UP) per well of a flat-bottom 96-well plate. Include a NF-κB positive control (e.g. recombinant hTNF-α at 1 ng/ml), an IRF positive control (e.g. recombinant hIFN-β at 1x10^4 U/ml), and a negative control (e.g. endotoxin free water).

Note: Use new tips for each well to avoid cross-contamination.

2. Add 180 µl of cell suspension (~100,000 cells) per well.
3. Incubate the plate for 18-24 h at 37 °C, 5% CO_2.

Detection of NF-κB induction

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

Detection of IRF induction

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 enclosed TDS.
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. Add 10 µl of stimulated cell supernatant/well into a 96-well white (opaque) or black plate, or a luminometer tube.
4. Prime the injector with QUANTI-Luc™ 4 Reagent working solution and proceed with the measurement.

RELATED PRODUCTS

Product	Cat. Code
THP1-Dual™	thpd-nfis
THP1-Dual™ KO-TRIF	thpd-kotrif
THP1-Dual™ KO-TLR4	thpd-kotlr4
THP1-Dual™ MD2-CD14-TLR4	thpd-mctlr4
THP1-Dual™ MD2-CD14 KO-TLR4	thpd-mckotlr4
Recombinant human TNF-α	rcyc-htnfa
Recombinant human IL-1β	rcyec-hil1b
C12-iE-DAP	tlrl-c12dap
Pam3CSK4	tlrl-pms
Flagellin-ST Ultrapure (UP)	tlrl-epstfla-5
LPS-EB UP	tlrl-3pelps
PMA	rcyec-hil1b
QUANTI-Blue™ Solution	rep-qbs1
QUANTI-Luc™ 4 Lucia/Gaussia	rep-qlc4lg1
Normocin™	ant-nr-1
Blasticidin	ant-bl-1
Zeocin®	ant-zn-1

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

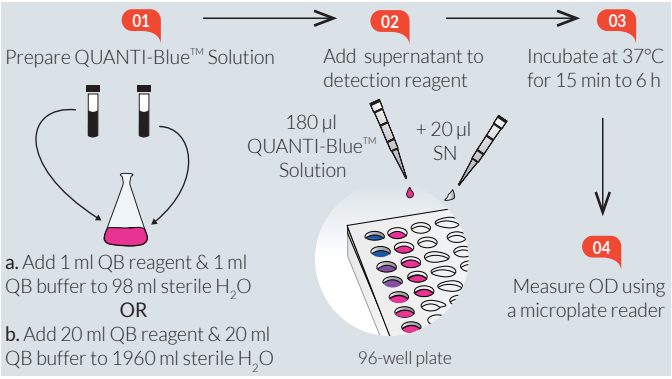


Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com

B. High Throughput Screening (HTS) procedure

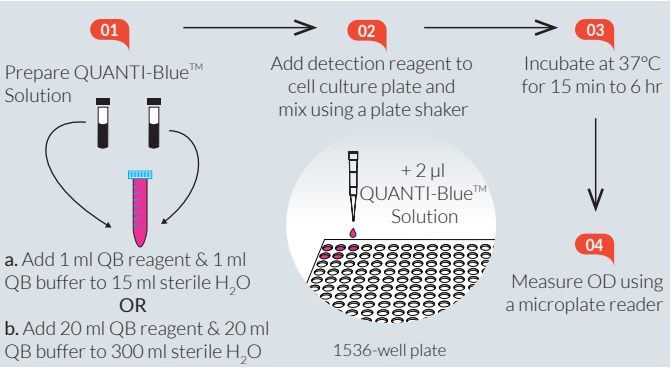


Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

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 23C24-AK

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 kit.

QUANTI-Luc™ 4 Lucia/Gaussia comprises two liquid components:

- QUANTI-Luc™ 4 Reagent 20X (coelenterazine substrate)
- QUANTI-Luc™ 4 Stabilizer 25X (optimized Glow assay reagent)

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 a component of the QUANTI-Luc™ 4 Lucia/Gaussia kit. 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).

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 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 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	
500 tests	rep-qlc4lg1
2 x 500 tests	rep-qlc4lg2
5 x 500 tests	rep-qlc4lg5

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 Asia: +852 3622-3480
E-mail: info@invivogen.com