HEK-Blue™ IL-1β Cells

IL-1β Reporter Cells

Catalog code: hkb-il1b

https://www.invivogen.com/hek-blue-il1b

For research use only

Version 20F09-MM

PRODUCT INFORMATION

Contents:

- 3-7 x 10^6 HEK-Blue™ IL-1β 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 Hygromycin B Gold (>90% pure hygromycin B) at 100 mg/ml. Store at 4 °C or -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 three antibiotics active against mycoplasmas, bacteria, and fungi. Store 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.

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 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. HEK-Blue™ IL-1β cells should not be passaged more than 20 times to remain fully efficient.

Quality Control

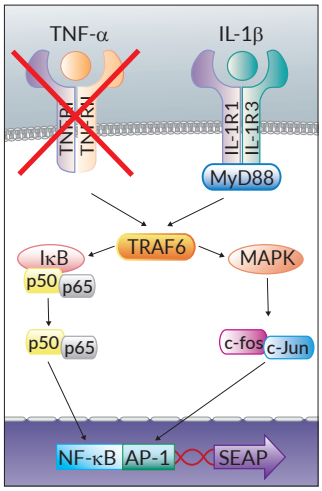
- SEAP reporter activity in response to IL-1β and other cytokines has been validated using functional assays.
- The stability for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

USE RESTRICTIONS

These cells are distributed for research purposes only.

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.

BACKGROUND



Interleukin-1β (IL-1β) is a secreted pro-inflammatory cytokine that plays a critical role in the host response to infection and injury[1]. It derives from a pro-protein that is produced by activated macrophages and cleaved by caspase-1[2]. The resulting mature IL-1β is secreted and binds to the IL-1R1 receptor. This triggers the formation of the IL-1R1/IL-1R3/MyD88 complex and induces signaling leading to the activation of the transcription factors NK-κB and AP-1 with the subsequent inflammatory response[3]. Due to its role in mediating acute and chronic inflammation, IL-1β has emerged as a therapeutic target for auto-inflammatory diseases [4].

1. Dinarello C., 2018. Overview of the IL-1 family in innate inflammation and acquired immunity. Immunol Rev. 281(1): 8–27. **2. Lopez-Castejon G. & Brough D., 2011**. Understanding the mechanism of IL-1β secretion. Cytokine Growth Factor Rev. 22(4):189-95. **3. WeberA. *et al.*, 2010.** Interleukin-1 (IL-1) pathway. Sci Signal. 3(105):cm1. **4. Dinarello CA., 2011.** Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. Blood. 117:3720–3732.

PRODUCT DESCRIPTION

HEK-Blue™ IL-1β cells allow the detection of bioactive IL-1β by monitoring the activation of the NF-κB and AP-1 pathways. They can also detect IL-1α, as both cytokines bind to the same receptor, IL-1R1. Of note, in these human embryonic kidney 293 (HEK293)-derived cells, the response to human TNF-α has been blocked while the response to murine TNF-α remains intact.

These cells express a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of the IFN-β minimal promoter fused to five NF-κB and five AP-1 binding sites. Binding of IL-1 to its receptor IL-1R1 on the surface of HEK-Blue™ IL-1β cells triggers a signaling cascade leading to the activation NF-κB/AP-1 and the subsequent production of SEAP. Detection of SEAP in the supernatant of HEK-Blue™ IL-1β cells can be assessed using QUANTI-Blue™ Solution, a SEAP detection medium.

Detection range

Human IL-1α/β: 100 pg - 100 ng/ml
Murine IL-1α/β: 30 ng - 1 µg/ml

The specificity of the HEK-Blue™ IL-1β cells for the detection of IL-1α or IL-1β can be confirmed using a neutralizing antibody against human IL-1α or IL-1β, such as anti-hIL-1α-IgG and anti-hIL-1β-IgG. HEK-Blue™ IL-1β cells are resistant to hygromycin B and Zeocin™.

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

InvivoGen
www.invivogen.com

SAFETY CONSIDERATIONS

HEK-Blue™ IL-1β 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% (v/v) heat-inactivated fetal bovine serum (FBS; 30 min at 56 °C), 100 U/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml Normocin™
- **Test Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% (v/v) heat-inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin **without Hygromycin B Gold, Normocin™, and Zeocin™**
- **Freezing Medium:** DMEM with 20% (v/v) FBS and 10% (v/v) DMSO

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 in 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 T-25 culture flask containing 5 ml of growth medium.

Note: To avoid excessive alkalinity of the medium during recovery of the cells, place the tissue culture flask containing the growth medium into the incubator for at least 15 minutes prior to the addition of the vial contents.

7. Place the culture 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 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. Aliquot 1 ml of 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. Maintain and subculture the cells in growth medium with 100 µg/ml of Zeocin™ and 200 µg/ml of Hygromycin B Gold.
2. Renew growth medium twice a week.
3. Cells should be passaged when a 70-80% confluency is reached, detach the cells in presence of PBS by tapping the flask or by using a cell scraper. Do not let the cells grow to 100% confluency.

Note: The response of HEK-Blue™ IL-1β cells can be altered by the action of trypsin. Do not use trypsin to detach HEK-Blue™ IL-1β cells.

APPLICATION

HEK-Blue™ IL-1β cells are useful to monitor IL-1β in inflammasome activation studies. THP-1 human monocytic cells represent the most commonly used model cell line for the study of inflammasome activation. To become susceptible to inflammasome inducers, these cells must be induced by stimuli commonly used for induction in model systems, such as lipopolysaccharide (LPS) and phorbol 12-myristate acetate (PMA). Stimulation by LPS or differentiation with PMA induces the production of pro-IL-1β, the immature form of IL-1β. Subsequent stimulation with inflammasome inducers, such as ATP and alum crystals, leads to caspase-1 activation and IL-1β maturation and secretion.

Stimulation of HEK-Blue™ IL-1β cells with supernatants of THP-1 cells treated with LPS or PMA and stimulated with inflammasome inducers triggers the production of SEAP that correlates with the presence of IL-1β in the supernatants (see THP-1/HEK-Blue™ IL-1β Assay). Co-incubation with a monoclonal anti-IL-1β antibody blocks the response (see Related Products).

DETECTION OF IL-1β IN THP-1 SUPERNATANTS

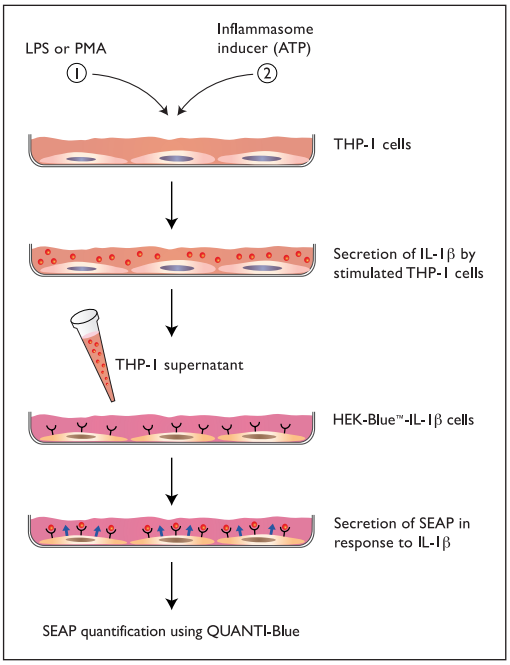

Figure 2. THP-1/HEK-Blue™ IL-1β Assay

THP-1 Activation

THP-1 cells are grown in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum, 2 mM L-glutamine, Pen/Strep, Normocin™. THP-1 cells are grown in suspension to a density of 1 x 10^6 cells/ml in tissue culture flasks.

• Option 1: PMA induction

Day 1

1. Add 180 µl of THP-1 cell suspension per well of a 96-well plate (2 x 10^6 cells/well).
2. Treat THP-1 cells with 20 µl of phorbol myristate acetate (PMA; final concentration 20-50 ng/ml) for 3 hours at 37 °C in 5% CO_2.
3. Wash cells gently with pre-warmed PBS and add 200 µl supplemented RPMI.

Day 4

4. Wash cells with pre-warmed PBS and add 180 µl supplemented RPMI.
5. Add 20 µl of an inflammasome inducer, such as ATP or alum hydroxide (see Related Products).
6. Incubate overnight at 37 °C in 5% CO_2.

Note: The production of pro-IL-1β can be further increased by priming PMA-activated THP-1 cells with LPS (follow protocol below).

• Option 2: LPS induction

1. Prepare a THP-1 cell suspension at 2 x 10^6 cells/ml and add 180 µl of this cell suspension per well of a 96-well plate (3 x 10^5 cells/well).
2. Treat THP-1 cells with 20 µl of LPS (final concentration 1 µg/ml) for 3 hours at 37 °C in 5% CO_2.
3. Remove gently medium and add 180 µl supplemented RPMI.
4. Add 20 µl of an inflammasome inducer, such as ATP or alum hydroxide.
5. Incubate overnight at 37 °C in 5% CO_2.

Detection of IL-1β by HEK-Blue™ IL-1β cells

Day 1

1. Prepare HEK-Blue™ IL-1β cell suspension: gently rinse cells twice with pre-warmed PBS, detach the cells in presence of PBS by tapping the flask or by using a cell scraper, resuspend cells in fresh, pre-warmed test medium (which contains heat-inactivated FBS) and prepare a cell suspension at 3 x 10^5 cells/ml.

Notes:

- 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.

- The response of HEK-Blue™ IL-1β cells can be altered by the action of trypsin. Do not use trypsin to detach HEK-Blue™ IL-1β cells.

2. Add 50 µl of activated THP-1 supernatant per well of a flat-bottom 96-well plate.
3. In separate wells, add 50 µl of recombinant human IL-1β at 0.25 µg/ml, as the positive control, and 50 µl of recombinant human TNF-α at 0.25 µg/ml, as a negative control.

Note: HEK-Blue™ IL-1β cells should not respond to human TNF-α .

4. Add 150 µl of HEK-Blue™ IL-1β cell suspension (~50,000 cells) per well.
5. Incubate overnight at 37 °C in 5% CO_2.

Day 2

6. Prepare QUANTI-Blue™ Solution following the instructions on the enclosed product data sheet.
7. Add 180 µl of resuspended QUANTI-Blue™ Solution per well of a flat-bottom 96-well plate.
8. Add 20 µl of induced HEK-Blue™ IL-1β cells supernatant.
9. Incubate the plate at 37 °C for 30 min to 3 hours.
10. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Description	Cat.Code
Alum Hydroxide	Inflammasome inducer	tlrl-aloh
Anti-hIL-1α-IgG	Neutralizing antibody	mabg-hil1a-3
Anti-hIL-1β-IgG	Neutralizing antibody	mabg-hil1b-3
ATP	Inflammasome inducer	tlrl-atpl
Hygromycin B Gold	Selection antibiotic	ant-hg-1
LPS-EK	TLR4 agonist	tlrl-eklps
Normocin™	Antimicrobial reagent	ant-nr-1
PMA	NF-κB Activator	tlrl-pma
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
Recombinant human IL-1β	Recombinant cytokine	rcyec-hil1b
Recombinant human TNF-α	Recombinant cytokine	rcyc-htnfa
Zeocin™	Selection antibiotic	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 20C16-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. If this occurs, vortex the product 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 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 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

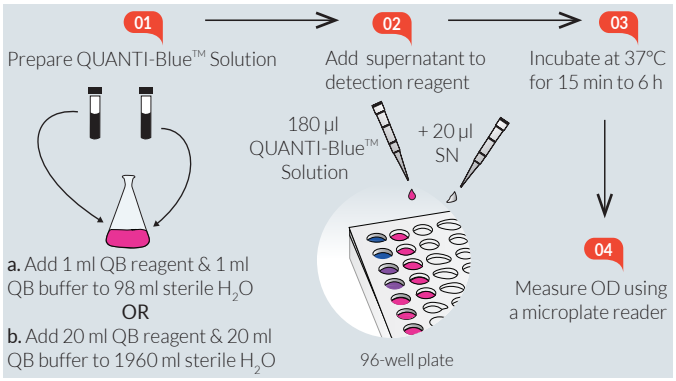

Figure 1. Standard procedure using QUANTI-Blue™ Solution.

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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E-mail: info@invivogen.com

B. High Throughput Screening (HTS) procedure

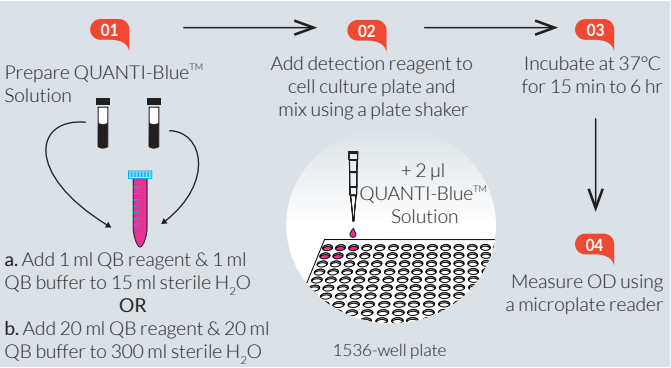

Figure 2. High throughput screening procedure using QUANTI-Blue™ Solution.

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