PD-1/PD-L1 Bio-IC™

Anti-immune checkpoint cell-based assay

Catalog code: rajkt-hpd1

https://www.invivogen.com/hpd1-bioassay

For research use only

Version 23J17-NJ

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 of Jurkat-Lucia™ TCR-hPD-1 cells in a cryovial or shipping flask.
- 3-7 x 10^6 of Raji-APC-hPD-L1 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 Hygromycin** (100 mg/ml). Store at 4 °C or at -20 °C.*
- **1 ml of G418 (Geneticin)** (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 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.

Notes:

- QUANTI-Luc™ 4 Reagent is photosensitive and should be protected from light.

- Data sheets for all components are available on our website.

Handling Cells Upon Receipt

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: **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 cells more than 20 times and maintain cells in growth medium supplemented with corresponding selective antibiotics.

Quality Control

- Human CD28, PD-1, and PD-L1 expression has been verified by flow-cytometry.
- Reporter activity has been validated using InvivoGen's anti-hPD-1 and anti-hPD-L1 hIgG1 antibodies.
- The stability for 20 passages following thawing has been verified.
- Both cell lines are guaranteed mycoplasma-free.

1. Shaw J-P. *et al.*, 1998. Identification of a putative regulator of early T cell activation genes. Science. 241:202-205. 2. Wei S.C. *et al.*, 2018. Fundamental mechanisms of immune checkpoint blockade therapy. Cancer Discovery. 8(9):1069-86. 3. Ribas A. and Wolchock J.D., 2018. Cancer immunotherapy using checkpoint blockade. Science. 359:1350-55.

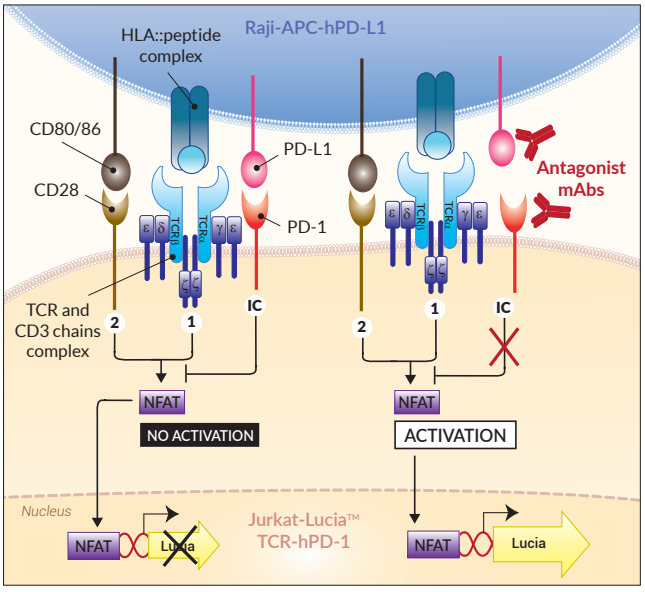
PRODUCT DESCRIPTION

PD-1/PD-L1 Bio-IC™ is a bioluminescent cell-based assay designed for the screening of novel inhibitors of the PD-1/PD-L1 immune checkpoint (IC) axis. The assay consists of two engineered cell lines:

- **Jurkat-Lucia™ TCR-hPD-1 effector cells** were engineered from the human T-lymphocyte Jurkat cell line which naturally expresses a functional NFAT pathway[1]. Jurkat-Lucia™ TCR-hPD-1 cells stably express a specific [HLA::peptide]-restricted TCR and the Lucia luciferase reporter gene under the control of an ISG54 minimal promoter fused to six NFAT response elements. In addition, these cells overexpress human (h) CD28 stimulatory receptor[2] and hPD-1 (programmed cell death 1; aka CD279) inhibitory receptor[3].

- **Raji-APC-hPD-L1 cells** were engineered from the human B lymphocyte-derived Raji cell line. These cells were stably transfected to express a specific [HLA::peptide] and overexpress hPD-L1 (programmed cell death ligand 1; aka CD274 or B7-H1)[3]. Raji cells are characterized by a number of cell-surface expressed markers including the B cell receptor (BCR), CD19, and CD20. Additionally, they naturally express various ICs including CD27, CD70, CD80.

These cells have been functionally tested with various Anti-hPD-1 or Anti-hPD-L1 hIgG1 mAbs. Upon addition of blocking mAbs, the PD-1/PD-L1 inhibitory interaction is disrupted and Jurkat-Lucia™ TCR-hPD-1 effector cells express Lucia luciferase in a dose-dependent manner. Please, see next page for each cell line resistance to selective antibiotics.



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 2: Raji-APC-hPD-L1 cells were derived from Raji cells, which contain Herpesvirus (EBV), and thus may require Biosafety Level 2. Please check with your country's regulatory authority regarding the use of these cells.

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** IMDM, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated fetal bovine serum (FBS; 30 min at 56 °C), 100 µg/ml Normocin™, Pen-Strep (100 U/ml-100 µg/ml)
- **Freezing Medium:** 90% FBS, 10% DMSO
- **Test Medium:** IMDM, 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated FBS, Pen-Strep (100 U/ml-100 µg/ml) **without Normocin, Blasticidin, Zeocin™, Hygromycin, and G418 (Geneticin).**

Required Selective Antibiotics

- **Jurkat-Lucia™ TCR-hPD-1 cells:** Blasticidin, Zeocin™, Hygromycin, and G418 (Geneticin).
- **Raji-APC-hPD-L1 cells:** Blasticidin and G418 (Geneticin).

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 must 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 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 cells at 150 x g (RCF) for 10 mins.
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.
7. Place the culture at 37 °C in 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10^6 cells/ml in freezing medium freshly prepared with cold FBS.
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. Both cell lines grow in suspension.
2. After cells have recovered, subculture:

- **Jurkat-Lucia™ TCR-hPD-1 cells** in growth medium with an initial seeding density of ~300,000 cells/ml. To maintain selection pressure, add 10 µg/ml of Blasticidin, 100 µg/ml of Zeocin™, 100 µg/ml Hygromycin, and 250 µg/ml of G418 (Geneticin) every other passage.
- **Raji-APC-hPD-L1 cells** in growth medium with an initial seeding density of ~200,000 cells/ml. To maintain selection pressure, add 10 µg/ml of Blasticidin and 250 µg/ml of G418 (Geneticin) every other passage.

3. Renew growth medium twice a week.

Cell-Handling Recommendations

To ensure the best results:

- Use cells with less than 20 passages.
- Handling of cells should be as short as possible to prevent any damage resulting from the prolonged stay at room temperature without 5% CO_2.

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 info@invivogen.com.

APPLICATION

InvivoGen's PD-1/PD-L1 Bio-IC™ has been designed to measure the potency of antibody-, Fc-fusion protein-, or small molecule-based inhibitors of the PD-1/PD-L1 axis.

Below is a protocol to perform a blocking assay with Anti-hPD-1-Ni-hIgG1 monoclonal antibody (mAb) in a standard flat-bottom 96-well plate.

Antibody Preparation

1. Prepare dilutions of test mAb using 1X PBS (phosphate buffered saline). Include a positive control (e.g. Anti-hPD-1-Ni-hIgG1) and a negative control (e.g. Anti-β–Gal-hIgG1).

Note: We recommend to prepare a 1:2 dilution series.

2. Add 20 µl of test and control mAbs per well.

Cell Preparation

Day-2:

1. Centrifuge cells at 300 x g (RCF) for 5 min.
2. Remove supernatant and resuspend cells in pre-warmed test medium:

- Jurkat-Lucia™ TCR-hPD-1 cells at 5 x 10^5 cells/ml
- Raji-APC-hPD-L1 cells at 4 x 10^5 cells/ml

Day 0:

1. Centrifuge cells at 300 x g (RCF) for 5 min.
2. Remove supernatant and resuspend cells in pre-warmed test medium:

- Jurkat-Lucia™ TCR-hPD-1 cells at 2.2 x 10^6 cells/ml
- Raji-APC-hPD-L1 cells at 1.1 x 10^6 cells/ml

Note: Raji-APC-Null cells can be used as control APCs. They allow TCR and CD28, but not PD-1 engagement in Jurkat-Lucia™ TCR-hPD-1 cells.

Note: To ensure reproducible results, homogenize the cell suspensions.

Reporter assay

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

1. Add 90 µl of Jurkat-Lucia™ TCR-hPD-1 cell suspension (~200,000 cells) and 90 µl of Raji-APC-hPD-L1 or Raji-APC-Null cell suspension (~100,000 cells) per well containing test/control mAbs.
2. Incubate the plate at 37 °C in a CO_2 incubator for 6 h.
3. Prepare QUANTI-Luc™ 4 Reagent working solution following the instructions on the data sheet.
4. Transfer 20 µl of co-cultured cell supernatant into a 96-well white (opaque) or black plate, or a luminometer tube.
5. Add 50 µl of QUANTI-Luc™ 4 Reagent working solution per well.
6. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-05
Zeocin®	Selection antibiotic	ant-zn-05
Hygromycin	Selection antibiotic	ant-hg-1
G418 (Geneticin)	Selection antibiotic	ant-gn-1
QUANTI-Luc™ 4 Lucia/Gaussia	Luminescence detection kit	rep-qlc4lg1

QUANTI-Luc™ 4 Reagent

A coelenterazine-based luminescence assay reagent

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

For research use only

Version 23F27-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 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).

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

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