

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 21E04-NJ

PRODUCT INFORMATION

Contents and Storage

- 1 vial of Jurkat-Lucia™ TCR-hPD-1 cells (3-7 x 10⁶ cells)
- 1 vial of Raji-APC-hPD-L1 cells (3-7 x 10⁶ cells)

IMPORTANT: Cells are shipped frozen. If cells 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). Normocin™ is a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store 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 or for 1 month at -20°C. Keep reconstituted QUANTI-Luc™ away from light.

*The expiry date is specified on the product label.

Note: 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 hlgG1 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. 3.

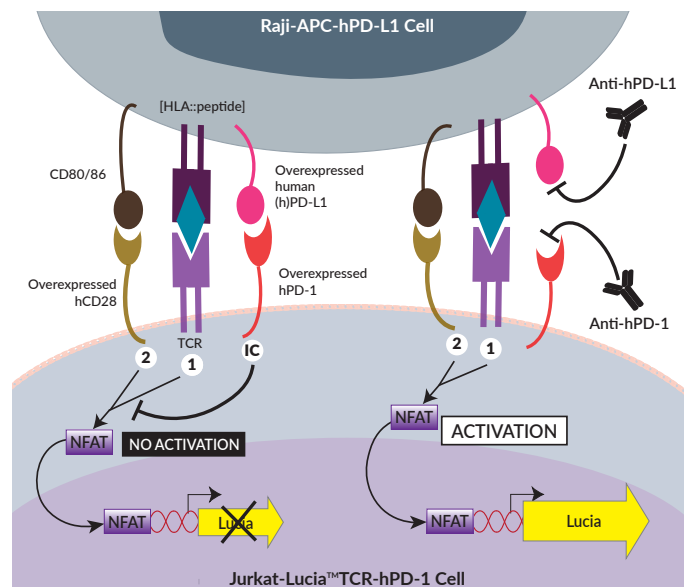
PRODUCT DESCRIPTION

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 pathway¹. 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 receptor² and hPD-1 (programmed cell death 1; aka CD279) inhibitory receptor³.

- **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 hlgG1 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

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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, Blastidicin, Zeocin™, Hygromycin, and G418 (Geneticin).

Required Selective Antibiotics

- **Jurkat-Lucia™ TCR-hPD-1 cells:** [Blastidicin](#), [Zeocin™](#), [Hygromycin](#), and [G418 \(Geneticin\)](#).
- **Raji-APC-hPD-L1 cells:** [Blastidicin](#) 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% CO₂.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10⁶ 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 [Blastidicin](#), 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 [Blastidicin](#) 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% CO₂.

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-hlgG1](#) 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-hlgG1](#)) and a negative control (e.g. [Anti-β-Gal-hlgG1](#)).
Note: We recommend to prepare a 1:2 dilution series.
2. Add 20 µl of test and control mAbs per well.

Cell Preparation

- Note: Passage cells at indicated concentrations 4 days prior to the assay.*
1. Centrifuge cells at 300 x g (RCF) for 5 mins.
 2. Remove supernatant and resuspend cells in fresh, pre-warmed test medium:
 - Jurkat-Lucia™ TCR-hPD-1 cells at 2.2 x 10⁶ cells/ml
 - Raji-APC-hPD-L1 cells at 1.1 x 10⁶ 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 CO₂ incubator for 6 h.
3. Prepare [QUANTI-Luc™](#) 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™](#) per well.
6. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Description	Cat. Code
Anti-β-Gal-hlgG1	Control antibody	bgal-mab1
Anti-hPD-1-Ni-hlgG1	Anti-hPD-1 antibody	hpd1ni-mab1
Blastidicin	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™	Lucia detection medium	rep-qlc1

TECHNICAL SUPPORT

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