(PD-1/CTLA4)/PD-L1 Bio-IC™

Anti-immune checkpoint cell-based assay

Catalog code: rajkt-ctla4-pdl1

invivogen.com/immune-checkpoint-hpd1-hctla4-hpdl1-bioassay

For research use only

Version 25F23-NJ

PRODUCT INFORMATION

Contents and Storage

- **3-7 x 10^6 of Jurkat-Lucia™ TCR-hCTLA4-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.

Note: 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 Puromycin** (10 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. QUANTI-Luc™ 4 Reagent is photosensitive and should be protected from light.

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

Handling Frozen Cells Upon Arrival

Cells are shipped in dry ice, and upon receipt should immediately be thawed for culture or stored below -130°C, preferably in liquid nitrogen vapor, for long-term storage.

IMPORTANT: Do not store cell vials at -80°C as this will decrease cell viability and performance. Contact technical support if the cells are not frozen or in dry ice upon arrival.

To insure the highest level of viability and best assay performance, we strongly recommend that you thaw the cells and initiate the culture as soon as possible upon receipt (as described on the next page).

Warranties

- InvivoGen's cells are provided 'AS IS' and their viability is guaranteed upon shipment from our facilities for a period of 30 days, provided that the customer has properly stored and handled the product.
- Our cell lines are guaranteed free of mycoplasma contamination.
- The stability of our cell lines is guaranteed for 20 passages.

Quality Control

- Human PD-1, CTLA4, CD28, PD-L1, and CD80 expression has been verified by flow-cytometry.
- Reporter activity is validated using InvivoGen's Anti-hPD-1 and Anti-hCTLA4 monoclonal antibodies (mAbs).
- Our cell lines are guaranteed free of mycoplasma contamination.
- The stability of our cell lines is guaranteed for 20 passages.

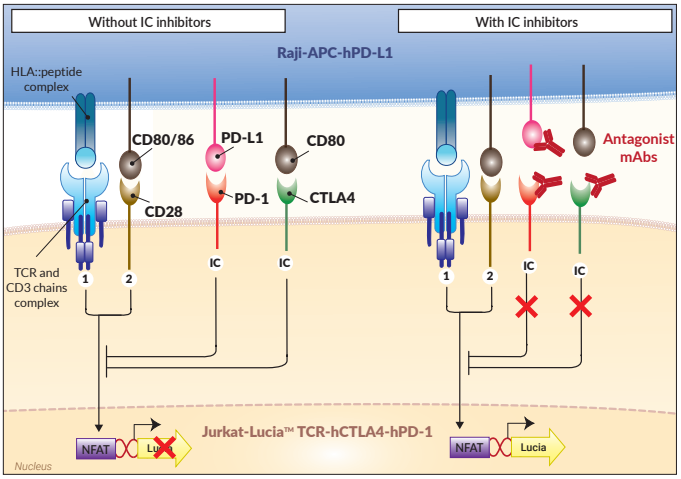
PRODUCT DESCRIPTION

(PD-1/CTLA4)/PD-L1 Bio-IC™ is a bioluminescent cell-based assay designed for the exploration of combination therapies that target both PD-1/PD-L1 and CTLA4/CD80 immune checkpoint (IC) axes[1]. The assay relies on the co-culture of two cell lines:

- **Jurkat-Lucia™ TCR-hCTLA4-hPD-1 effector cells:** They were engineered from the human T-lymphocyte Jurkat cell line which naturally expresses a functional NFAT pathway[2]. Jurkat-Lucia™ TCR-hCTLA4-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) PD-1 (programmed cell death 1, CD279) inhibitory receptor, as well as hCD28 stimulatory and hCTLA4 inhibitory receptors that share the hCD80 ligand[3].

- **Raji-APC-hPD-L1 cells:** They 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 2, CD274, B7-H1)[3]. Raji cells are characterized by a number of cell-surface expressed markers including the B cell receptor (BCR), hCD19, and hCD20. Additionally, they naturally express various ICs including hCD80, hCD27, and hCD70.

Upon addition of blocking Anti-hPD-1 or Anti-hCTLA4 mAbs, the IC inhibitory interaction is disrupted and Jurkat-Lucia™ TCR-hCTLA4-hPD-1 effector cells express Lucia luciferase in a dose-dependent manner. The combination of Anti-hPD-1 or Anti-hCTLA4 mAbs results in stronger inhibition than when each antibody is used alone. Please, see next page for each cell line resistance to selective antibiotics.



1. Lisi L. *et al.*, 2022. Clinical experience with CTLA-4 blockade for cancer immunotherapy: From the monospecific monoclonal antibody ipilimumab to probodies and bispecific molecules targeting the tumor microenvironment. Pharmacol Research 175:105997. 2. Shaw J-P. *et al.*, 1998. Identification of a putative regulator of early T cell activation genes. Science. 241:202-205. 3. Ribas A. and Wolchock J.D., 2018. Cancer immunotherapy using checkpoint blockade. Science. 359:1350-55.

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

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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, Puromycin, and G418 (Geneticin).**

Required Selective Antibiotics

- **Jurkat-Lucia™ TCR-hCTLA4-hPD-1 cells:** Blasticidin, G418 (Geneticin), Hygromycin, Puromycin, and Zeocin®.
- **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 min.
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-hCTLA4-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 5 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

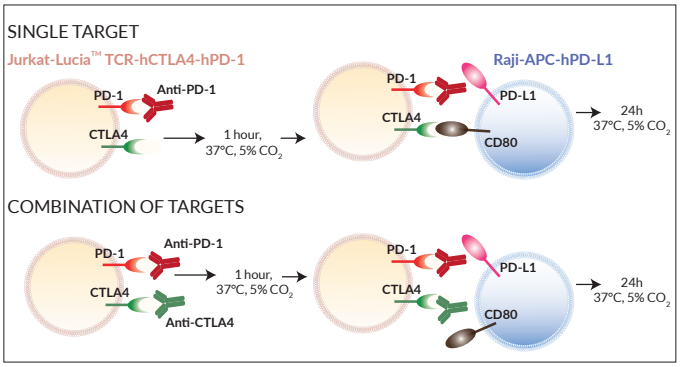
These cells are distributed for research purposes only. This product is covered by a Limited Use License. By use of this product, the buyer agrees to the terms and conditions of all applicable Limited Use Label Licenses. For non-research use, such as screening, quality control or clinical development, contact outlicensing@invivogen.com.

APPLICATION

InvivoGen's (PD-1/CTLA4)/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 and/or CTLA4/CD80 axes.

Below is a protocol to perform a blocking assay with Anti-hPD1-Ni-hIgG4 (S228P) and Anti-hCTLA4-hIgG1 monoclonal antibodies (mAbs), either alone or in combination, in a standard flat-bottom 96-well plate.

Principle of target blocking with single or combination of antibodies



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 or Anti-β–Gal-hIgG4 (S228P).

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

2. Add 20 µl of each test or control mAbs per well.

Note: In wells with no combination of two blocking mAbs, but only one, add 20 µl of pre-warmed test medium instead of the second mAb.

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-hCTLA4-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-hCTLA4-hPD-1 cells at 1.25 x 10^6 cells/ml
- Raji-APC-hPD-L1 cells at 6.25 x 10^5 cells/ml

Note: Raji-APC-Null cells can be used as control APCs. They allow strong TCR and CD28, and low PD-1 engagement in Jurkat-Lucia™ TCR-hPD-1 cells.

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

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Co-culture assay

Assay using one blocking mAb:

1. Antibody and cell line incubation:
   - If targeting PD-1 **or** CTLA4, add 80 µl of Jurkat-Lucia™ TCR-hCTLA4-hPD-1 cell suspension (~100,000 cells) per well containing test/control mAbs.
   - If targeting PD-L1, add 80 µl of Raji-APC-hPD-L1 (~50,000 cells) per well containing test/control mAbs.

   Note: In wells with no combination of two blocking mAbs, but only one, add 20 µl of pre-warmed test medium instead of the second mAb.
2. Incubate the plate at 37 °C in a CO_2 incubator for 1h.
3. Co-culture of both cell lines:
   - If targeting PD-1 **or** CTLA4, add 80 µl of Raji-APC-hPD-L1 (~50,000 cells) to the wells containing the Jurkat-Lucia™ TCR-hCTLA4-PD-1 and blocking mAb.
   - If targeting PD-L1, add 80 µl of Jurkat-Lucia™ TCR-hPD-1 (~100,000 cells) to the wells containing the Raji-APC-hPD-L1 and blocking mAb.
4. Incubate the plate at 37 °C in a CO_2 incubator for 24h.

Assay using two blocking mAbs:

1. Antibody and cell line incubation:
   Add 80 µl of Jurkat-Lucia™ TCR-hCTLA4-hPD-1 cell suspension (~100,000 cells) per well containing Anti-hCTLA4 **and** Anti-hPD-1, or control mAbs.
2. Incubate the plate at 37 °C in a CO_2 incubator for 1h.
3. Add 80 µl of Raji-APC-hPD-L1 (~50,000 cells) to the wells containing the Jurkat-Lucia™ TCR-hCTLA4-hPD-1 and blocking mAbs
4. Incubate the plate at 37 °C in a CO_2 incubator for 24h.

Reporter assay

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

1. Prepare QUANTI-Luc™ 4 Reagent working solution following the instructions on the data sheet.
2. Transfer 20 µl of co-cultured cell supernatant into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Add 50 µl of QUANTI-Luc™ 4 Reagent working solution per well.
4. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Cat. code
Anti-hCTLA4-hIgG1	hctla4-mab1
Anti-hCTLA4-hIgG1fut	hctla4-mab13
Anti-hCTLA4-hIgG4 (S228P)	hctla4-mab14
Anti-hPD-1-Ni-hIgG4 (S228P)	hpd1ni-mab114
Anti-hPD-L1-hIg1 (N298A)	hpdl1-mab12
Zeocin®	ant-zn-05
Quanti-Luc™ 4 Lucia/Gaussia	rep-qlc4lg1

QUANTI-Luc™ 4 Reagent

A coelenterazine-based luminescence assay reagent

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

For research use only

Version 24G30-MM

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).

Note: Lucia® is a registered trademark of InvivoGen.

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

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