4-1BB/4-1BBL Bio-IC™

Anti-immune checkpoint cell-based assay

Catalog code: rajkt-41bb

https://www.invivogen.com/h41bb-bioassay

For research use only

Version 24J07-MM

PRODUCT INFORMATION

Contents and Storage

- **3-7 x 10^6 of Jurkat-Lucia™ h4-1BB cells** in a cryovial or shipping flask.
- **3-7 x 10^6 of Raji-Null 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 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:

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

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

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 4-1BB and 4-1BBL expression is assessed by flow cytometry.
- The bioassay is validated using an anti-h4-1BB antagonist antibody.
- The stability for 20 passages following thawing is confirmed.
- These cells are tested for mycoplasma contamination.

1. Gonzales A.M, and Orlando R.A., 2009. A Jurkat transcriptional reporter cell line for high-throughput analysis of the nuclear factor-kappaB signaling pathway. N. Biotechnol. 26(5):244-50. 2. Bartkowiak, T. & Curran, M.A. 2015. 4-1BB Agonists: Multi-Potent Potentiators of Tumor Immunity. Front Oncol 5, 117.

PRODUCT DESCRIPTION

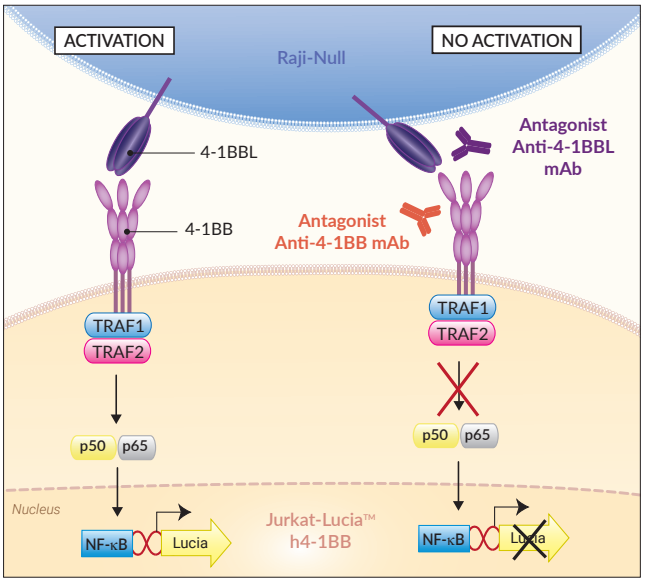
4-1BB/4-1BBL Bio-IC™ is a bioluminescent cell-based assay designed for the screening of novel inhibitors of the 4-1BB/4-1BBL immune checkpoint (IC) axis. The assay consists of two engineered cell lines:

- **Jurkat-Lucia™ h4-1BB effector cells** were engineered from the human T-lymphocyte Jurkat cell line which naturally expresses a functional NF-κB pathway[1]. Jurkat-Lucia™ h4-1BB cells stably express human 4-1BB (aka CD137 or TNFSF9) at the plasma membrane, as well as an NF-κB-inducible Lucia luciferase reporter gene. This ensures the triggering of the TRAF1-TRAF2-NF-κB signaling pathway upon 4-1BB and 4-1BBL interaction[2]. These cells are resistant to Blasticidin and Zeocin®.

- **Raji-Null cells** were engineered from the human B lymphocyte-derived Raji cell line. Raji cells naturally express 4-1BBL (CD137L), the ligand for 4-1BB, as well as other various ICs including CD80 and PD-L1. These cells are resistant to Blasticidin.

These paired cell lines have been functionally tested with an anti-h4-1BB blocking monoclonal antibody (mAb). In absence of the mAb, the 4-1BB/4-1BBL activatory interaction triggers Lucia luciferase expression in the Jurkat-Lucia™ h4-1BB effector cells. In presence of the mAb, the 4-1BB/4-1BBL interaction is disrupted and the effector cells do not express Lucia luciferase.

Note: If you are interested in a model that allows the mimicking of an immune synapse through T cell receptor (TCR) crosslinking with [HLA::peptide] complexes, along with the 4-1BB-4-1BBL costimulatory interaction, please contact us.



TECHNICAL SUPPORT

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FAQ Any questions about our cell lines?

Visit our FAQ page.

InvivoGen

www.invivogen.com

SAFETY CONSIDERATIONS

Biosafety Level 2: Raji-Null cells were derived from Raji cells, which contain Herpesvirus (EBV), and thus may require Biosafety Level 2. The biosafety level varies by country. 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, and Zeocin®.**

Required Selective Antibiotics

- **Jurkat-Lucia™ h4-1BB cells:** Blasticidin, and Zeocin®.
- **Raji-Null cells:** Blasticidin.

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™ h4-1BB cells** in growth medium with an initial seeding density of ~300,000 cells/ml. To maintain selection pressure, add 10 µg/ml of Blasticidin, and 100 µg/ml of Zeocin® every other passage.
- **Raji-Null cells** in growth medium with an initial seeding density of ~200,000 cells/ml. To maintain selection pressure, add 10 µg/ml of Blasticidin 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 4-1BB/4-1BBL Bio-IC™ has been designed to measure the potency of antibody-, Fc-fusion protein-, or small molecule-based inhibitors of the 4-1BB/4-1BBL axis.

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

Day -2:

Cell Preparation

1. Centrifuge Jurkat-Lucia™ h4-1BB and Raji-Null cells at 300 x g (RCF) for 5 min.
2. Resuspend cells in fresh, pre-warmed test medium:

- Jurkat-Lucia™ h4-1BB cells: 5 x 10^5 cells/ml
- Raji-Null cells: 4 x 10^5 cells/ml

Day 0:

Antagonist Antibody Preparation

1. Prepare dilutions of test mAb using 1X PBS (phosphate buffered saline). Include a positive control (e.g. Anti-h4-1BB-rIgG2a) and a negative control (e.g. Anti-β-Gal-rIgG2a).

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

2. Add 20 µl of test and control mAbs per well of a flat-bottom 96-well plate.

Cell Preparation

1. Centrifuge Jurkat-Lucia™ h4-1BB and Raji-Null cells at 300 x g (RCF) for 5 min.
2. Resuspend cells at 1.1 x 10^6 cells/ml in fresh, pre-warmed test medium.

Note: To ensure reproducible results, use a pipet to homogenize the cell suspensions.

3. Add 90 µl (~100,000 cells) of Jurkat-Lucia™ h4-1BB cell suspension per well containing test/control mAbs.
4. Incubate the plate at 37 °C in a CO_2 incubator for 1 h.
5. Add 90 µl (~100,000 cells) of Raji-Null cell suspension per well.
6. Incubate the plate at 37 °C in a CO_2 incubator for 24 h.

Reporter read-out

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 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	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-05
Zeocin®	Selection antibiotic	ant-zn-05
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 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

TECHNICAL SUPPORT
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