Raji-APC-Null Cells

Activating control APC for anti-immune checkpoint cell-based assay

Catalog code: raji-apc-null

https://www.invivogen.com/raji-apc-null

For research use only

Version 21E04-NJ

PRODUCT INFORMATION

Contents and Storage

- 3-7 x 10^6 Raji-APC-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 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.*

*The expiry date is specified on the product label.

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

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: **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.

IMPORTANT: For cells that arrive in a shipping flask please refer to the enclosed 'cell recovery procedure'.

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 the selective antibiotic.

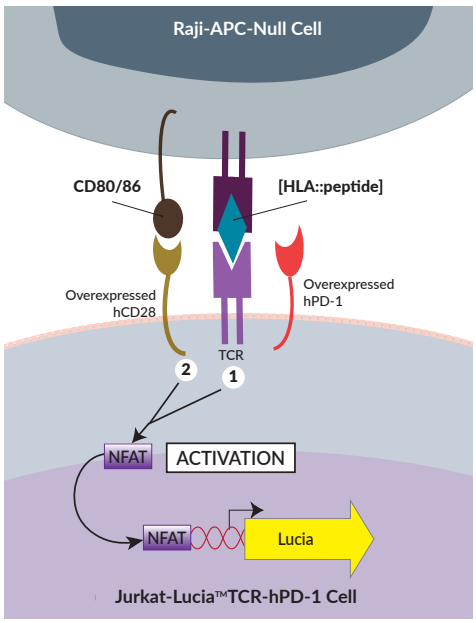
Quality Control

- Activation of Jurkat-Lucia™ TCR-hPD-1 reporter cell line using Raji-APC-Null cells as antigen presenting cells has been validated.
- The stability for 20 passages following thawing has been verified.
- Raji-APC-Null cells are guaranteed mycoplasma-free.

PRODUCT DESCRIPTION

Raji-APC-Null cells were engineered from the human B lymphocyte-derived Raji cell line. They were stably transfected to express a specific [HLA::peptide] complex, providing a cognate TCR stimulation on Jurkat-Lucia™ TCR-hPD-1 cells. Raji-APC-Null cells naturally express CD80/86 molecules which provide a co-stimulatory signal upon interaction with CD28 on the effector cells. 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 immune checkpoint (IC) molecules, including CD27, CD70, CD80.

Raji-APC-Null cell line's resistance to Blasticidin and G418 allows them to be used with the same selection pressure than Raji-APC-hPD-L1 target cells.



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.

APPLICATION

Raji-APC-Null cells have been designed as a **control antigen presenting cell line** for InvivoGen's PD-1/PD-L1 Bio-IC™. They do not provide PD-L1 inhibitory interaction to co-cultured Jurkat-Lucia™ TCR-hPD-1 effector cells, which thus express the NFAT-inducible Lucia luciferase reporter at maximal levels following TCR activation.

For more information, visit https://www.invivogen.com/hpd1-bioassay.

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.

SAFETY CONSIDERATIONS

Biosafety Level 2

Raji-APC-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), Pen-Strep (100 U/ml-100 µg/ml), 100 µg/ml Normocin™
- **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 G418 (Geneticin)**

Required Selective Antibiotics

- 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. Raji-APC-Null cells grow in suspension.
2. After cells have recovered, subculture 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 Raji-APC-Null 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.

APPLICATION

PROTOCOL FOR PD-1/PD-L1 Bio-IC™

For more information, visit https://www.invivogen.com/hpd1-bioassay

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 of a standard flat-bottom 96-well plate.

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 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™ 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-hIgG1	Control antibody	bgal-mab1
Anti-hPD-1-Ni-hIgG1	Anti-hPD-1 antibody	hpd1ni-mab1
Blasticidin	Selection antibiotic	ant-bl-05
Geneticin	Selection antibiotic	ant-gn-1
QUANTI-Luc™	Lucia detection medium	rep-qlc1