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Lieferung & Zahlungsart

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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The PBMC Cytotoxicity Luciferase Assay Kit (Ramos) is designed to determine the cytotoxicity profile of PBMCs (Peripheral Blood Mononuclear Cells) towards the eGFP/Firefly Luciferase Ramos (RA 1) cell line. It uses the luminescent signal from the eGFP/Firefly Luciferase Ramos cell line to measure the number of live target cells within a mixed cell population of PBMC and eGFP/Firefly Luciferase Ramos cells. The kit contains PBMCs, the eGFP/Firefly Luciferase Ramos cell line, cell culture media and ONE-Step™ Luciferase Assay System. In addition, this kit includes a positive control bispecific antibody.

Background

Lymphocyte-mediated cytotoxicity is a form of cellular immunity against intracellular pathogens, including viruses and certain bacteria and parasites. The most popular *in vitro* methods to monitor lymphocyte-mediated cytotoxicity on target cells are cell-mediated cytotoxicity assays such as ADCC (antibody-dependent cellular cytotoxicity) and TDCC (T-cell dependent cellular cytotoxicity) in which immune effector cells and target cells are co-cultured. To analyze immune effector cell cytolytic activity in such heterogeneous cell population of effector and target cells, it is important to be able to discriminate between effector and target cell populations with distinct phenotypes. The use of luciferase allows for a clear separation between the effector and the surviving target cells. The instability of firefly luciferase when released from dead cells in cell culture gives it a half-life of approximately 2 hours, minimizing any residual luminescence signal generated from dead target cells. Cytotoxicity assays are crucial to understand the potency of CAR (chimeric antigen receptor) T and NK cells, and antibody-based immunotherapies.

Ramos is a human Burkitt's lymphoma cell line grown in suspension. The eGFP/Firefly Luciferase Ramos cell line is a Ramos cell line expressing firefly luciferase and enhanced GFP (eGFP) driven by an EF1a promoter. Luciferase activity is directly proportional to live cell numbers.

Application(s)

- Luciferase- based analysis of live and dead target cells in cytotoxicity assays.
- Test the efficacy of multi-specific immune engager molecules.
- Assess the Fc effector function of candidate antibodies.

Supplied Materials

Catalog #	Name	Amount	Storage
79059	Normal Human Peripheral Blood Mononuclear Cells, Frozen	2 vials at 10 x 10^6 cells each	Liquid Nitrogen
82149	eGFP/ Firefly Luciferase Ramos (RA 1) Cell Line	2 vials at >1 million cells each	Liquid Nitrogen
79652	Thaw Medium 8	1 x 100 ml	4°C
60184	Thaw Medium 2 (Assay Medium)	2 x 100 ml	4°C
82153	Growth Medium 8B	1 x 100 ml	4°C
60690-1	ONE-Step™ Luciferase Assay System	2 x 10 ml kit	-20°C
100836	Anti-CD20-Anti-CD3 Bispecific Antibody	1 x 20 ug	-20°C

Materials Required but Not Supplied

- 96 Well White, Clear Bottom Plate
- T75 cell culture flask
- Luminometer

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

- This protocol is a general guideline only.
- This protocol is designed to perform cytotoxicity assays in a 96-well plate. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- Each vial of human PBMCs (effector cells) is sufficient for 60 wells of a 96-well plate at an effector to target cell ratio (E: T) of 10:1 ($1x10^5$ effector cells: $1x10^4$ target cells). For a higher ratio of E: T cell ratio you may need to thaw both PBMC vials supplied.
- eGPF/Firefly Luciferase Ramos Cell Line (#82149) maintenance conditions can be found on the cell line product page, eGFP/Firefly Luciferase Ramos cell line (bpsbioscience.com).
- The antibody dilution range should be optimized for your assay. A starting concentration of 50- 500 nM is recommended as the highest value in the preparation of 5x antibody dilutions.
- We recommend the use of the following experimental controls:
    - Control 1: No antibody control. This control contains both PBMCs and target cells without antibody. This control is used to measure the maximum luminescence signal in the assay.
    - Control 2: PBMCs cells only. This control is used to determine the background luminescence signal.
    - Control 3: Negative antibody control. This control contains both effector and target cells in the presence of serial dilutions of a non-specific antibody (antibody of the same class and isotype as the specific antibody but unable to recognize the target).
    - Control 4: Positive antibody control. Anti-CD20-Anti-CD3 Bispecific Antibody is provided as a positive control antibody.

Target Cell Thaw and Expansion (one week prior to running the assay)

Cell Thawing

1. Retrieve a Ramos cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw medium 8 to the conical tube containing the cells. Thaw medium 8 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 8.

5. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

6. After 24 hours of culture, check for viability. For a T25 flask, add 3-4 ml of fresh Thaw Medium 8 and continue growing culture in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

7. Cells should be passaged before they reach 2 x 10^6 cells/ml. At first passage and subsequent passages, use Growth Medium 8B.

Cell Passage
Passage cells at least once to make sure they are healthy (2 x 10^6 cells are needed for the assay described below) by diluting the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, but no less than 0.2 x 10^6 cells/ml, in Growth Medium 8B**.** The sub-cultivation ratio should maintain the cells between 0.2 x 10^6 cells/ml and 2 x 10^6 cells/ml.

Day 1: PBMC Cell Preparation

1. Thaw one vial of PBMCs by swirling the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 2.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Spin down at 300 *x g* for 5 minutes, aspirate supernatant, and resuspend cell pellet in 10 ml of Thaw Medium 2 (1 x 10^6 cells/ml).

3. Plate cells in a T75 flask in Thaw Medium 2.

4. Incubate the flask overnight in a humidified 37°C incubator with 5% CO_2.

   Note: This step will enrich the lymphocyte population by depleting adherent cells.

Day 2: Assay
For 96-well plate assays, each well will contain a final volume of 125 µl (25 µl of 5x antibody dilution, 50 µl of PBMCs at desired E:T ratio and 50 µl of Firefly Luciferase RA 1 cells as target cells).

1. Transfer 2 x 10^6 eGFP/Firefly Luciferase Ramos cells to a clean 15 ml tube and centrifuge at 300 *x g* for 5 minutes.

2. Aspirate supernatant and resuspend eGFP/Firefly Luciferase Ramos (RA 1) cells in 10 ml of Thaw Medium 2
   (2 x 10^5 cells/ml).

3. Transfer cells to a solution reservoir.

4. Using a multichannel pipette, transfer 50 µl of eGFP/Firefly Luciferase Ramos (RA 1) cell suspension (10,000 cells/well) to the test antibody, Control 1, Control 3 and Control 4 wells.

5. Using a multichannel pipette, transfer 50 µl of Thaw Medium 2 to the Control 2 wells.

6. Keep the plate in a humidified 37°C incubator with 5% CO_2 while preparing PBMCs.

7. Collect PBMCs into a 15 ml tube and count cells.

   Note: Be careful to avoid detachment of the adherent cells by not shaking the T75 flask prior to or while transferring cells.

8. Centrifuge PBMCs at 300 *x g* for 5 minutes and aspirate the supernatant.

9. Dilute PBMCs in Thaw Medium 2 to 2 x 10^6 cells/ml.

   Note E:T ratio may need to be optimized in different experimental settings and cell density may need to be adjusted.

10. Add 50 µl of PBMC suspension to the test antibody, Control 1, Control 2, Control 3 and Control 4 wells.

11. Keep the plate in a humidified 37°C incubator with 5% CO_2 while you are preparing antibody dilutions.

12. Prepare test antibody and antibody control dilutions at 5x the final concentrations to be tested, in Thaw Medium 2 (25 µl/well), starting at 500 nM.

13. Add 25 µl of the test antibody dilutions to the Test antibody wells.

14. Add 25 µl of the antibody control dilutions to the Control 3 and Control 4 wells.

15. Add 25 µl of Thaw Medium 2 to Control 1, and Control 2 wells.

16. Incubate the assay plate for 24 hours in a humidified 37°C incubator with 5% CO_2.

    Note: The incubation time may need to be optimized for your assay.

Example of Plate Schematic:

	1	2	3	4	5	6	7	8	9	10	11	12	
A	Dilu12	Dilu11	Dilu10	Dilu9	Dilu8	Dilu7	Dilu6	Dilu5	Dilu4	Dilu3	Dilu2	Dilu1	Test antibody
B	Dilu12	Dilu11	Dilu10	Dilu9	Dilu8	Dilu7	Dilu6	Dilu5	Dilu4	Dilu3	Dilu2	Dilu1	
C	Dilu12	Dilu11	Dilu10	Dilu9	Dilu8	Dilu7	Dilu6	Dilu5	Dilu4	Dilu3	Dilu2	Dilu1	Control 3
D	Dilu12	Dilu11	Dilu10	Dilu9	Dilu8	Dilu7	Dilu6	Dilu5	Dilu4	Dilu3	Dilu2	Dilu1	
C	Dilu12	Dilu11	Dilu10	Dilu9	Dilu8	Dilu7	Dilu6	Dilu5	Dilu4	Dilu3	Dilu2	Dilu1	Control 4
F	Dilu12	Dilu11	Dilu10	Dilu9	Dilu8	Dilu7	Dilu6	Dilu5	Dilu4	Dilu3	Dilu2	Dilu1	
G	Control 1	Control 2											
H	Control 1	Control 2											

Day 3: Luciferase Analysis

1. Thaw Luciferase Reagent Component A by placing the reagent in a Room Temperature (RT) water bath.

2. Equilibrate Component A to RT and mix well before use.

3. Immediately before the experiment, prepare the Luciferase Assay Working Solution by diluting Luciferase Reagent Substrate (Component B) 100-fold with Luciferase Component A, and mix well (you will need 125 µl/well).

   Note: Avoid exposure to excessive light. Only use enough of each component for the experiment, and store the remaining Component A and Component B separately at -20°C.

4. Remove the cells from the incubator and add 125 µl of Luciferase Assay Working Solution directly to the culture medium of each well.

5. Wrap the plate with foil and gently rock it for ≥15 minutes at RT.

6. Measure firefly luminescence using a luminometer.

Example Results

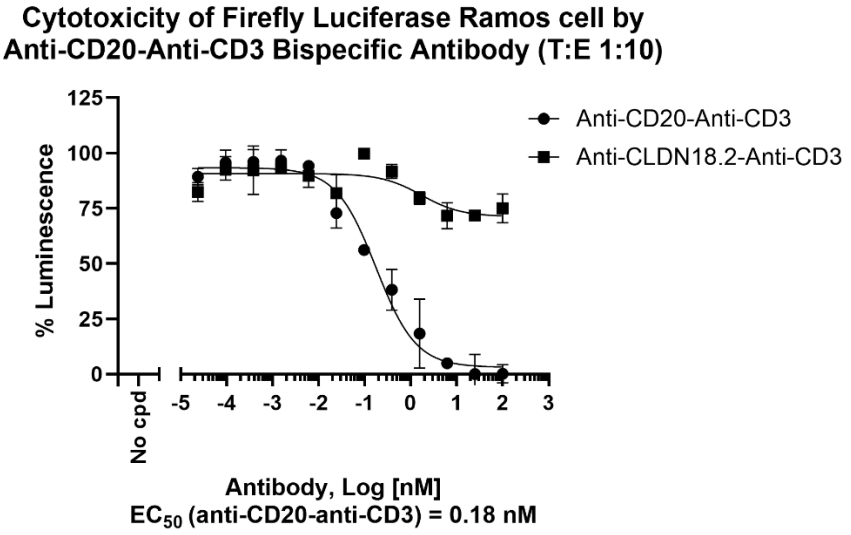

Figure 1. Cytotoxicity of the eGPF/Firefly Luciferase Ramos (RA 1) Cell Line triggered by the Anti-CD20-Anti-CD3 Bispecific Molecule.

PBMCs and eGFP/Firefly Luciferase Ramos (RA 1) cells were combined at a 10:1 ratio in a 96-well white, clear bottom plate. The cells were incubated with a dilution series of Anti-CD20-Anti-CD3 Bispecific Antibody (#100836) or the negative antibody control, Anti-CLDN18.2-Anti-CD3 Bispecific Antibody (#101541), in a humidified 37°C incubator with 5% CO_2 for 24 hours. After incubation, luciferase activity was measured with ONE-Step™ Luciferase reagent. The raw luminescence data were fitted to a sigmoidal three-parameter curve using GraphPad Prism® software.

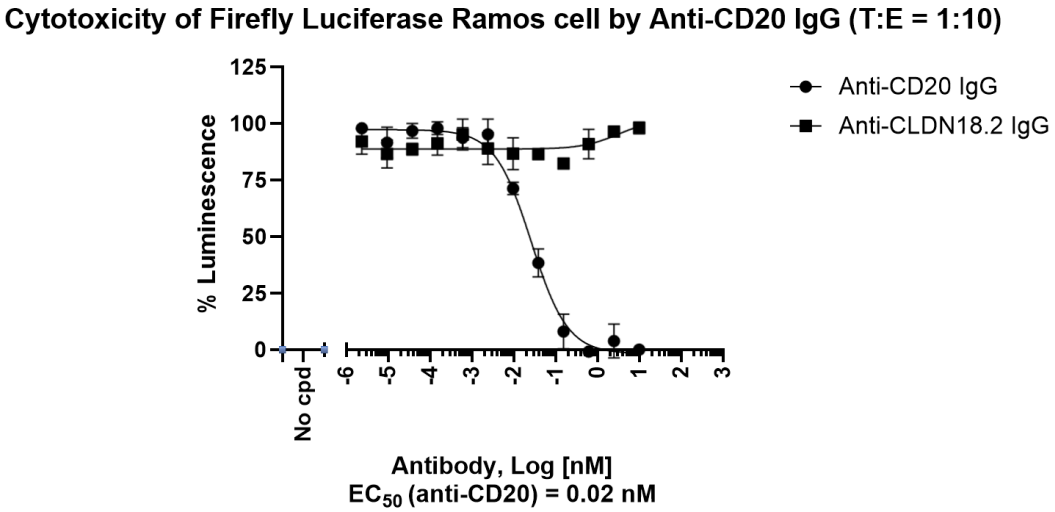

Figure 2. Cytotoxicity of the eGPF/Firefly Luciferase Ramos (RA 1) Cell Line triggered by the Anti-CD20 antibody.

PBMCs and eGPF/Firefly Luciferase Ramos (RA1) cells were combined at a 10:1 ratio in a 96-well white, clear bottom plate. The cells were incubated with a dilution series of Anti-CD20 IgG (#71209) or the negative control antibody, Anti-Claudin-18 Isoform 2 IgG Antibody (#101564), in a humidified 37°C incubator with 5% CO_2 for 24 hours. After incubation, luciferase activity was

measured with ONE-Step™ Luciferase reagent. The raw luminescence data were fitted to a sigmoidal three-parameter curve using GraphPad Prism® software.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

License Disclosure

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Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
PBMC Cytotoxicity Luciferase Assay Kit (NALM6)	82174	1 kit
PBMC Cytotoxicity Bioassay Kit (CFSE, 7-AAD)	82173	1 kit
PBMC Cytotoxicity Luciferase Assay Kit	82214	1 kit
Human T Cell Activation Reagent	82283	1x 10^8/ 1x 10^9
Human T Cell Isolation Kit	82288	1x 10^8
Anti-CD3 Antibody, FITC-Labeled	102008	25 µg/ 100 µg

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