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Description

The PBMC Cytotoxicity Luciferase Assay Kit (RPMI 8226) is a kit designed to determine the cytotoxicity profile of PBMCs (Peripheral Blood Mononuclear Cells) towards the Firefly Luciferase RPMI 8226 Cell Line. It uses the luminescence signal from Firefly Luciferase - RPMI 8226 Recombinant Cell Line to measure the number of live target cells within a mixed cell population of PBMC and Firefly Luciferase RPMI 8226 cells. The kit contains PBMCs, Firefly Luciferase – RPMI 8226 Recombinant Cell Line, cell culture media, and One-Step™ Luciferase Assay System. In addition, this kit includes a positive control Anti-BCMA-anti-CD3 bispecific molecule.

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 target cells in cell culture gives it a half-life of approximately 2 hours, eliminating 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.

RPMI 8226 cells are human B cells isolated from a plasmacytoma/myeloma patient. RPMI 8226 cells constitutively express BCMA and offer a physiologically relevant platform to evaluate cancer-directed immunotherapies, such as Chimeric Antigen Receptor (CAR) T cells. The Firefly Luciferase RPMI 8226 Recombinant Cell Line makes an excellent target to measure specific killing by CAR-T or NK cells targeting BCMA or other targets of interest. Luciferase activity is directly proportional to the number of alive cells.

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
79834	Firefly Luciferase – RPMI 8226 Recombinant Cell Line	2 vials at >1 million cells each	Liquid Nitrogen
60184	Thaw Medium 2 (Assay Medium)	1 x 100 ml	4°C
79704	Thaw Medium 10	1 x 100 ml	4°C
79835	Growth Medium 10A	1 x 100 ml	4°C
60690-1	ONE-Step™ Luciferase Assay System	2 x 10 ml kit	-20°C
100689	Anti-BCMA-Anti-CD3 Bispecific Molecule	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.
- Firefly Luciferase – RPMI 8226 Recombinant Cell Line should be cultivated in untreated culture flasks.
- 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 E:T cell ratio you may need to thaw both PBMC vials supplied.
- Firefly Luciferase – RPMI 8226 Recombinant Cell Line (BPS Bioscience #79834) maintenance conditions can be found at Firefly Luciferase – RPMI 8226 Recombinant Cell Line.
- 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 control Antibody: Anti-BCMA-Anti-CD3 Bispecific Molecule is used as the positive control antibody in our assay. We have made it available for customers to include it in their own assays if desired.

One week prior to running the assay: Target Cell Thaw and Expansion

Cell Thawing

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

2. Swirl 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 10.

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

3. 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 10.

4. Transfer the resuspended cells to a non-treated T25 flask and incubate at 37°C in a 5% CO_2 incubator.

5. After 24 hours of culture, check for viability. For a T25 flask, add 3-4 ml of fresh Thaw Medium 10 and continue growing culture in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

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

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 10A**.** 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 - RPMI 8226 cells as target cells).

1. Transfer 2 x 10^6 Firefly Luciferase RPMI 8226 cells to a clean 15 ml tube and centrifuge at 300 *x g* for 5 minutes.

2. Aspirate supernatant and resuspend Firefly Luciferase - RPMI 8226 cells in 10 ml of Assay Medium (Thaw Medium 2) at 2 x 10^5 cells/ml.

3. Transfer cells to a solution reservoir.

4. Using a multichannel pipette, transfer 50 µl of Firefly Luciferase - RPMI 8226 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 Assay Medium 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 Assay Medium 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 Control 1, Control 2, Control 3, Control 4, and Test Antibody wells.

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

12. Prepare Positive and Negative Control antibody dilutions at 5x the final concentrations to be tested, in Assay Medium (25 µl/well), starting at 50 nM.

13. Prepare test antibody dilutions at 5x the final concentrations to be tested, in Assay Medium (25 µl/well).

14. Add 25 µl of the Positive Control antibody dilutions to the Control 4 wells.

15. Add 25 µl of the Negative Control antibody dilutions to the Control 3 wells.

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

17. Add 25 µl of Assay Medium to Control 1, and Control 2 wells.

18. 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
B	Dilu12	Dilu11	Dilu10	Dilu9	Dilu8	Dilu7	Dilu6	Dilu5	Dilu4	Dilu3	Dilu2	Dilu1	Antibody
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	
E	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 Buffer (Component A) by placing the reagent in a Room Temperature (RT) water bath.

2. Equilibrate the buffer 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 Reagent Buffer (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

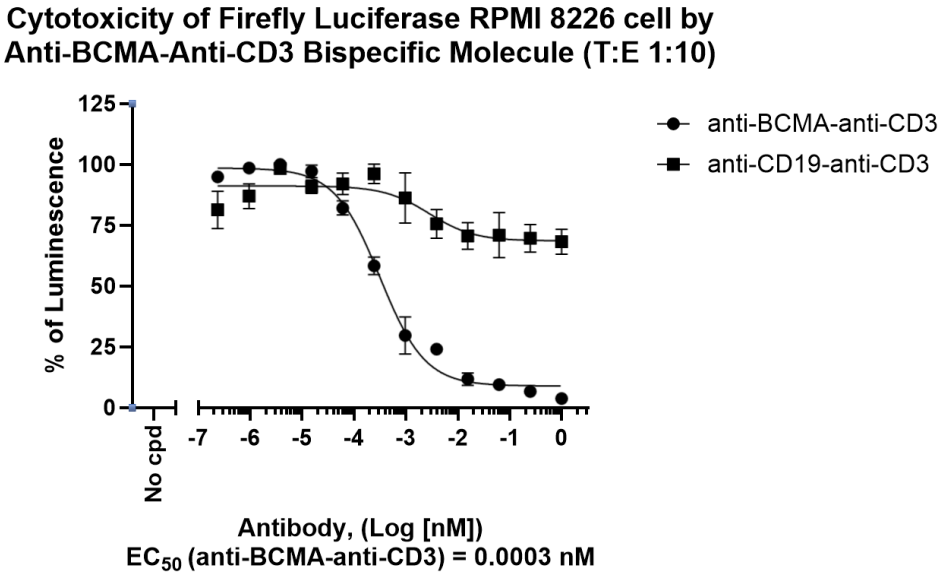

Figure 1. Cytotoxicity of the Firefly Luciferase - RPMI 8226 Recombinant Cell Line triggered by the Anti-BCMA-Anti-CD3 Bispecific Molecule.
PBMCs and Firefly Luciferase - RPMI 8226 cells were combined at E:T ratio of 10:1 in a 96-well white, clear bottom plate. The cells were incubated with a dilution series of Anti-BCMA-Anti-CD3 Bispecific Molecule (#100689) or the negative antibody control, Anti-CD19-Anti-CD3 Bispecific Molecule (#100441), 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.

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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 Luciferase Assay Kit (Ramos)	82694	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

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