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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The PBMC Cytotoxicity Bioassay Kit (CFSE, 7-AAD) is a kit designed to determine the cytotoxicity profile of PBMC (Peripheral Blood Mononuclear Cells) towards target cells of interest. It consists of CFSE (carboxyfluorescein succinimidyl ester), used to identify target cells in a mixed cell population of target cells and PBMC, and 7-AAD (7-aminoactinomycin D) to label dead cells. CFSE and 7-AAD labeling enables cell cytotoxicity detection at the single-cell level using flow cytometry. The kit also contains PBMCs, Thaw Medium and Assay Diluent.

Background

Lymphocyte-mediated cytotoxicity is a form of cellular immunity targeting intracellular pathogens, including viruses and certain bacteria and parasites. The most popular *in vitro* methods to monitor lymphocyte-mediated cytotoxicity of 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 populations it is essential to be able to discriminate between effector and target cell populations, with distinct phenotypes. The use of a label prior to co-culture with effector cells, like the membrane-permeable fluorescent dye CSFE (carboxyfluorescein succinimidyl ester), able to stain live target cells permits a clear distinction between live effector and target cells. After incubation with target cells, the DNA intercalating dye 7-ADD can be added to label dying target cells. These two dyes have distinct spectral properties and allow four cell populations to be discriminated by flow cytometry: live target cells ($CSFE^+7AAD^-$), dead target cells ($CSFE^+7AAD^+$), live effector cells ($CSFE^-7AAD^-$), and dead effector cells ($CSFE^-7AAD^+$). Cytotoxicity assays are crucial to understand the potency of CAR (chimeric antigen receptor) T and NK cells, and antibody-based immunotherapies.

Application(s)

Flow cytometry analysis of live target cells, dead target cells, live effector cells, and dead effector cells in cytotoxicity assays.

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
82177	500x CFSE	1 vial	-20°C
82178	1000x 7-ADD	1 vial	4°C
60184	Thaw Medium 2	2 x 100 ml	4°C
82184	Assay Diluent	25 ml	4°C

Materials Required but Not Supplied

- Target cells of interest.
- U-bottom 96 well plate for cell culture.
- T75 cell culture flask.

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. The E:T (effector:target) cell ratio and incubation time may need to be optimized, as it varies with the cell target of interest.
- The antibody dilution range should be optimized for your assay. A starting concentration of 100 nM is recommended in the preparation of 10X antibody dilutions.
- We recommend the use of the following controls for flow cytometer setting and compensation:
    - Control 1: Unstained target cells.
    - Control 2: CFSE single-stained target cells.
    - Control 3: 7-AAD single-stained target cells.
    - Control 4: No antibody control. This control contains both effector and target cells in the absence of an antibody (background cell death).
    - Control 5: 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 specific antibody but unable to recognize the target).

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

1. Thaw target cells.

2. Expand cells using the appropriate cell culture conditions for the cell line of interest.

3. Passage cells at least once to make sure they are healthy (4 x 10^6 cells are needed for the assay described below).

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.

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
Step1: Target Cell Labeling

1. Thaw 500x CFSE.
2. Prepare 0.5 ml of 1x CFSE Staining Solution by diluting 500x CFSE 500-fold with Assay Diluent.
3. Collect target cells, count, and prepare two 15 ml tubes with 2 x 10^6 cells each (4 x 10^6 cells needed).

   Note: One tube is going to be used to prepare CFSE-stained target cells, while the other will contain unstained target cells.

4. Spin down at 300 *x g* for 5 minutes.
5. Aspirate the supernatant and loosen the pellet by tapping the tubes.
6. Quickly resuspend cell pellet of one of the tubes (CFSE-stained target cell tube) in 200 µl of 1x CFSE Staining Solution (10^7 cells/ml).

   Note: A uniform suspension should be reached as quickly as possible as CFSE is taken up almost immediately and local variations in CFSE concentrations can affect staining uniformity.

7. Add 200 µl of Assay Diluent to the other 15 ml tube (control target cells without CFSE staining).
8. Incubate the cells for 15 minutes in a humidified 37°C incubator with 5% CO_2.
9. Add a minimum of 5 ml of Thaw Medium 2 to each tube.
10. Centrifuge at 300 *x g* for 5 minutes.
11. Aspirate the supernatant and resuspend the target cells in 5 ml of Thaw Medium 2.
12. Centrifuge the target cells at 300 *x g* for 5 minutes.
13. Aspirate the supernatant and resuspend target cells in 8 ml of Thaw Medium 2.
14. Transfer cell suspension to a solution reservoir.
15. Dilute cell density to 1.25 x 10^5 cells/ml by adding further 8 ml of Thaw Medium 2.
16. Mix gently.

17. Using a multichannel pipette, transfer 80 µl of CFSE-labeled target cell suspension (10,000 cells/well) to the U-bottom 96 plate wells corresponding to the test antibody, Control 2, Control 4, and Control 5.

18. Using a multichannel pipette, transfer 80 µl of non-labeled target cell suspension (10,000 cells/well) to the U-bottom 96 plate wells corresponding to the Control 1 and Control 3.

19. Incubate the cells in a humidified 37°C incubator with 5% CO_2 for 30 minutes.

Step 2: Cytotoxicity Assay

1. 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.

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

3. Dilute PBMCs with Thaw Medium 2 to 1 x 10^6 cells/ml.

4. Add 100 µl of PBMCs suspension to Test Antibody, Control 4, and Control 5 wells.

5. Add 100 µl of Thaw Medium 2 only to the Control 1, Control 2, and Control 3 wells.

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

7. Prepare an antibody serial dilution by making solutions 10x more concentrated than the concentrations to be tested, for both test and control antibodies (Control 5) (20 µl/well), starting at 100 nM.

8. Add 20 µl of test antibody dilutions to the test antibody wells.

9. Add 20 µl of control antibody dilutions to the Control 5 wells.

10. Add 20 µl of Thaw Medium 2 to the Control 1, Control 2, Control 3, and Control 4 wells.

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

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 5
D	Dilu12	Dilu11	Dilu10	Dilu9	Dilu8	Dilu7	Dilu6	Dilu5	Dilu4	Dilu3	Dilu2	Dilu1	
E	Control 1	Control 2	Control 3	Control 4									
F	Control 1	Control2	Control 3	Control 4									
G													
H													

Day 3: Flow Cytometry Analysis

1. Centrifuge the plate at 300 *x g* for 5 minutes.
2. Gently aspirate the supernatant.

   Note: Supernatants can be used to determine cytokine secretion.
3. Prepare 10 ml of 1x 7-AAD Staining Solution by diluting 1000x 7-ADD 1000-fold with Assay Diluent.
4. Resuspend Control 3, Control 4, and Control 5, and test antibody wells in 100 µl of 1x 7-AAD Staining Solution and mix well.
5. Resuspend Control 1 and Control 2 wells with 100 µl of Assay Diluent only.
6. Incubate the cells for 15 minutes in the dark at 4°C.
7. Analyze cells in a flow cytometer immediately.
8. Use appropriate controls for compensation.
9. Gate singlet cell populations on FSC-A vs FSC-H channels.
10. Gate $CFSE^+$ target cells (λexcitation/emission=488/525).
11. Determine 7-AAD positive (dead) and negative (positive) cells (λexcitation/λemission=488/647).

Example Results

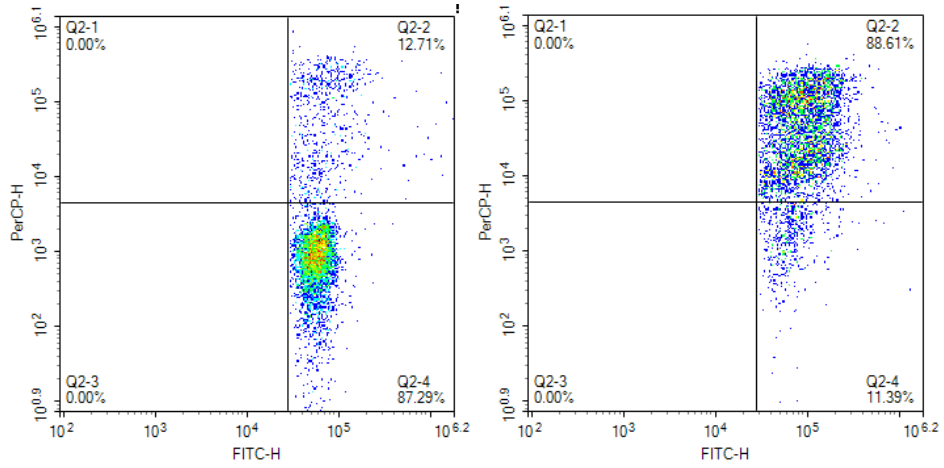

Figure 1: Flow cytometry analysis of CFSE-labelled NALM-6 target cells incubated with effector PBMCs.

CFSE-labelled NALM-6 cells were incubated with PBMCs at a 10:1 (E:T) cell ratio without (left) or with (right) Anti-CD19-Anti-CD3 Bispecific Molecule (BPS Bioscience #100441) for 24 hours, and stained with 7-AAD for 15 minutes. The $CFSE^+/7\text{-}AAD^+$ population corresponds to dying target cells (Q2-2 of the density plots).

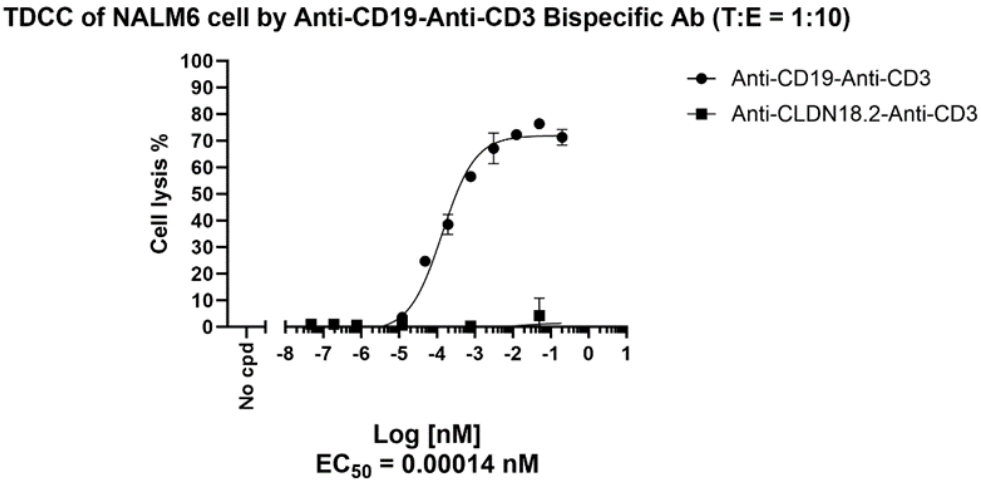

Figure 2. T cell-dependent cellular cytotoxicity (TDCC) of NALM6 cells triggered by the Anti-CD19-Anti-CD3 Bispecific Molecule.

PBMCs and CFSE-labeled NALM-6 cells were combined at a 10:1 ratio in a U-bottom plate. The cells were incubated with a dilution series of Anti-CD19-Anti-CD3 Bispecific Molecule (BPS Bioscience #100441) or the control Anti-CLDN18.2-Anti-CD3 Bispecific Antibody (BPS Bioscience #101541). After incubation for 24 hours in a humidified 37°C incubator with 5% CO_2, cells were stained with 7-AAD and analyzed by flow cytometry. The percentage of dying target cells ($CFSE^+$/ $7\text{-}AAD^+$ population) in each well was calculated. The raw cell death percentage data were fitted to a sigmoidal three-parameter curve using GraphPad Prism® software.

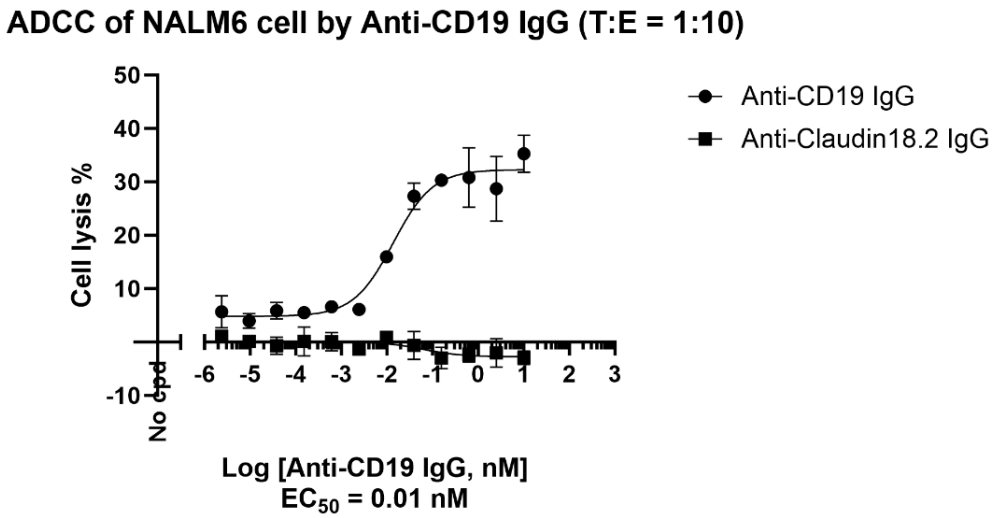

Figure 3. Antibody-dependent cellular cytotoxicity (ADCC) of NALM6 cells triggered by Anti-CD19 IgG.
Human PBMC and CFSE-labeled NALM-6 cells were combined at a 10:1 ratio in a U-bottom plate. The cells were incubated with a dilution series of Anti-CD19 IgG (BPS Bioscience #100981) or the control Anti-Claudin-18 Isoform 2 IgG Antibody (BPS Bioscience #101564). After incubation for 24 hours in a humidified 37°C incubator with 5% CO2, cells were stained with 7-AAD and analyzed by flow cytometry. The percentage of dying target cells ($CFSE^+/7\text{-}AAD^+$ population) in each well was calculated. The raw cell death percentage 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.

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
Firefly Luciferase NALM6 Cell Line	78494	2 vials
Anti-CD4 Antibody, PE-Labeled	102010	25 µg/100 µg
Anti-CD8 Antibody, PE-Labeled	102011	25 µg/100 µg
NCAM1/CD56 Positive Cell Isolation Kit	78808	1 x 10^8 Cells/1 x 10^9 Cells
Anti-NCAM1 Antibody, FITC-Labeled	101865	25 µg/100 µg

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