Description

The NK Cell Cytotoxicity Luciferase Assay Kit (NALM6) is designed to determine the cytotoxicity profile of NK (Natural Killer) cells towards the Firefly Luciferase NALM6 Cell Line. It uses the luminescence signal from Firefly Luciferase NALM6 Cell Line to measure the number of live target cells within a mixed cell population of NK and Firefly Luciferase NALM6 cells. The kit contains NK Cells, Firefly Luciferase NALM6 Cell Line, cell culture media and One-Step™ Luciferase Assay System.

Background

Lymphocyte-mediated cytotoxicity is a form of cellular immunity against intracellular pathogens, including viruses, certain bacteria, and parasites. The most commonly used *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 within this heterogeneous cell population of effector and target cells, it is important to discriminate between effector and target cell populations with distinct phenotypes. The use of luciferase allows for a clear separation between effector and 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 for understanding the potency of CAR (Chimeric Antigen Receptor) T cells, NK cells, and antibody-based immunotherapies.

NALM6 is a human B cell precursor leukemia cell line derived from the peripheral blood of a patient with acute lymphoblastic leukemia. The Firefly Luciferase NALM6 Cell Line constitutively expresses the Firefly (*Photinus pyralis*) luciferase reporter under the control of a CMV promoter. Luciferase activity is directly proportional to live cell numbers. These cells grow in suspension.

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
78798	Expanded Human Peripheral Blood NK Cells, Frozen	2 vials at 5 x 10^6 cells each	Liquid Nitrogen
78494	Firefly Luciferase NALM6 Cell Line	2 vials at >1 million cells each	Liquid Nitrogen
60184	Thaw Medium 2	2 x 100 ml	4°C
79639	Growth Medium 2D	1 x 100 ml	4°C
60690-1	ONE-Step™ Luciferase Assay System	2 x 10 ml kit	-20°C

Materials Required but Not Supplied

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

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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 NK cells (effector cells) is sufficient for 60 wells of a 96-well plate at an effector to target cell ratio (E: T) of 5:1 ($5x10^4$ effector cells: $1x10^4$ target cells). For a higher ratio of E: T cell ratio you may need to thaw both NK vials supplied.
- Firefly Luciferase NALM6 Cell Line (BPS Bioscience #78494) maintenance conditions can be found at Firefly Luciferase NALM6 Cell Line (bpsbioscience.com).
- The antibody dilution range should be optimized for your assay. A starting concentration of 50 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 NK cells and target cells without antibody. This control is used to measure the maximum luminescence signal in the assay.
    - Control 2: NK cells only. This control is used to determine the background luminescence signal.
    - Control 3: 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).

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

Cell Thawing

1. Retrieve a vial of NALM6 cells 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 2 to the conical tube containing the cells. Thaw Medium 2 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 2.

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 2 and continue growing culture in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

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7. Cells should be passaged before they reach 1 x 10^6 cells/ml. At first passage and subsequent passages, use Growth Medium 2D.

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 1 x 10^6 cells/ml, but no less than 0.2 x 10^6 cells/ml, in Growth Medium 2D**.** The sub-cultivation ratio should maintain the cells between 0.2 x 10^6 cells/ml and 1 x 10^6 cells/ml.

Day 1: NK Cell Preparation

1. Thaw one vial of NK cells 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.

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 NK cells at desired E:T ratio and 50 µl of Firefly Luciferase NALM6 cells as target cells).

1. Transfer 2 x 10^6 Firefly Luciferase NALM6 cells to a clean 15 ml tube and centrifuge at 300 *x g* for 5 minutes.

2. Aspirate supernatant and resuspend Firefly Luciferase NALM6 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 Firefly Luciferase NALM6 cell suspension (10,000 cells/well) to the test antibody, Control 1, and Control 3 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 NK cells.

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

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

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9. Dilute NK cells in Thaw Medium 2 to 1 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 NK cell suspension to the test antibody, Control 1, Control 2, and Control 3 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 50 nM.

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

14. Add 25 µl of the antibody control dilutions to the Control 3 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	
E	Control 1	Control 2											
F	Control 1	Control 2											
G													
H													

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.

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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

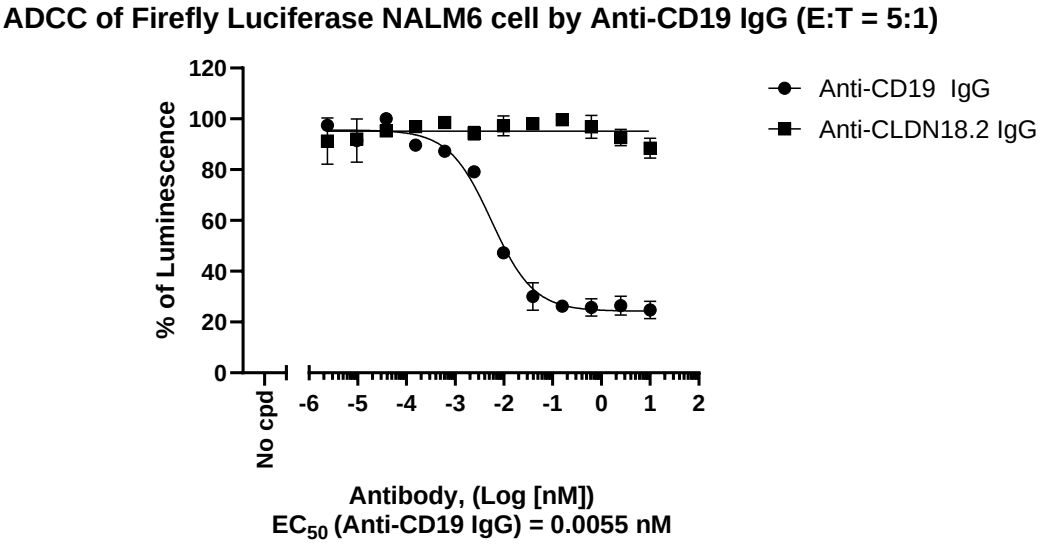

Figure 1. Antibody-dependent cellular cytotoxicity (ADCC) of Firefly Luciferase NALM6 Cell Line triggered by Anti-CD19 IgG.
NK cells and Firefly Luciferase NALM6 cells were combined at a 5:1 ratio in a 96-well white, clear bottom plate. The cells were incubated with a dilution series of Anti-CD19 IgG Antibody (#100981) or the negative control Anti-Claudin-18.2 IgG Antibody (101564). After incubation for 24 hours in a humidified 37°C incubator with 5% CO_2, 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

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Related Products

Products	*Catalog #*	*Size*
NK Cell Cytotoxicity Luciferase Assay Kit	82655	1 kit
PBMC Cytotoxicity Bioassay Kit (CFSE, 7-ADD)	82173	1 kit
PBMC Cytotoxicity Luciferase Assay Kit	82214	1 kit
Anti-NCAM1 Antibody, PE-Labeled	101673	25 µg/100 µg
Anti-NCAM1 Antibody, FITC-Labeled	101865	25 µg/100 µg
NCAM1 (CD56) CHO Cell Line (High, Medium or Low Expression)	78352	2 vials

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