Applied StemCell, Inc.
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Datasheet

iPSC-Derived Human NK Cell Starter Kit (African-American, Male Line)

Product Information

Catalog Number **ASE-9708**

Description

Applied StemCell has developed an efficient integration-free method to differentiate high-quality Natural Killer Cells (NK cells) from human iPSCs. The differentiated NK cells recapitulate the phenotype and functional parameters of primary and *in vivo* NK cells.

We provide NK cells differentiated from an integration-free, control human iPSC line (ASE-9211), reprogrammed from fibroblasts of an African-American male donor. These cryopreserved, high-purity (≥90%) cells express high levels of NK cells biomarkers, CD56 and CD45 (Figure 1), and the cytophagosis activity (Figure 2).

To harness the full potential of our NK cells, we also provide optimized NK Culture Media (ASE-9708MM) that supports the robust maintenance and functionality of the NK cells in culture.

These iPSC-differentiated NK cells can be used as control lines to compare phenotype and functionality of patient-derived, genome edited iPSC-derived NK cells and drug screening applications.

Parental Tissue

Control human iPSC (ASE-9211); p15
Age: Neonate
Gender: Male
Ethnicity: African-American
Tissue Source: Dermal Fibroblasts
Reprogramming Method: Episomal
Culture Conditions: Feeder-free

Clinical information

Healthy (with no known disease phenotypes)

Shipping

Dry ice

Storage and Stability

Store the components of the kit at the appropriate storage conditions as indicated in the media and materials table, immediately upon arrival. Shelf-life of the product is contingent upon proper storage conditions

Quality Control

Each lot of iPSC-derived human NK cells has been tested for growth, viability and purity (≥90%) following recovery from cryopreservation. In addition, each lot has been tested for expression of NK cell markers (CD45, CD56), and for the absence of mycoplasma and pathogens.

Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling frozen vials. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in

the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial.

Warranty

Performance of Applied StemCell's NK cells has been validated with the NK culture media provided in the NK Starter Kit and the recommended additional reagents. Applied StemCell will not hold responsibility if components other than the components provided with the NK Starter Kit and those recommended are used to culture the Applied StemCell NK cells.

Restricted Use

This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Characterization of the ASE-9708 NK Cells

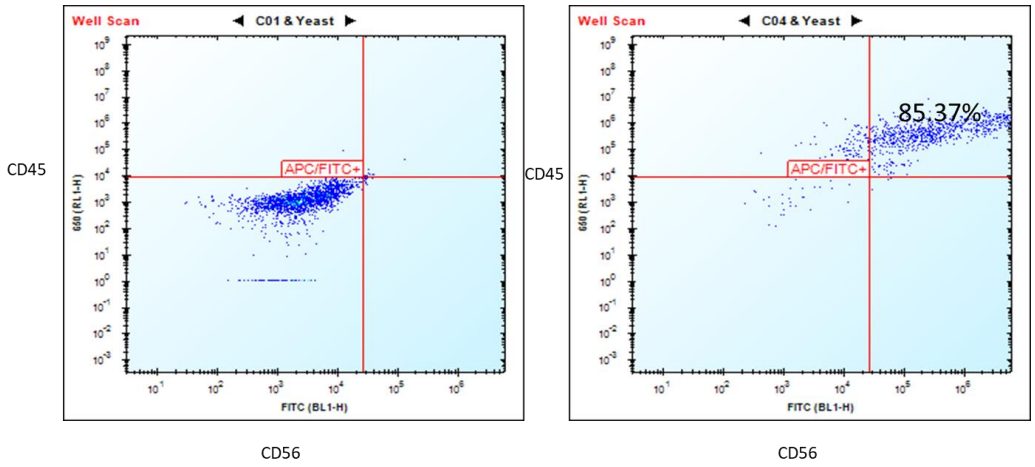

Figure 1. Flow cytometry analysis of ASE-9708 iPSC-derived NK cells for NK cell biomarkers. Cryopreserved NKs, differentiated from Applied StemCell's control iPSC line, ASE-9211 were recovered in NK culture media The cells were stained NK cell markers, CD45 and CD56 at day 2. Left: Isotype ctl antibodies. Right: CD45/CD56.

Media and Material

NK CellStarter Kit (ASE-9708)

Catalog #	Component	Amount	Storage	Shelf Life
ASE-9708-C	iPSC-derived NK cell; African-American Male Line	≥1 x 10^6 cells/ vial	Liq. N2	12 months
ASE-9708MM	NK Cell Culture Media	100 mL	-20°C	12 months
ASE-9708MM-A	NK Cell Media Supplement A (100x)	1 mL	-20°C	12 months

Additional Reagents Required

The below reagents are recommended for use with the motor neurons. If you use reagents other than those recommended, we suggest that you do a batch-test to validate integrity of the cells and culture protocol.

- Primary antibodies:
  - CD56 antibody (FITC): Biolegend, Cat# 362545
  - CD45 antibody (APC): Biolegend, Cat#368511
- Secondary antibodies: corresponding secondary antibodies were purchased from ThermoFisher

Protocol

1. Preparation of NK Cell Culture Media

1.1 Thaw the NK Cell Culture Media and supplement at room temperature before thawing the cryopreserved NKs.
1.2 Add 1mL NK cell media supplement A (100x) to 100mL NK cell Culture Media to make complete media.

1.3 The complete media should be aliquoted and stored at -20°C if it will not be used immediately.
Note: The complete media can be stored at 4°C for up to 2 weeks or at -20°C for up to 12 months.

2. Thawing and Culturing Cryopreserved NK

3.1 To thaw the cryopreserved NK cells, remove one vial from the storage unit.
3.2 Immerse the vial in the bath (up to 2/3rd of the vial) and thaw the cells rapidly until only a small piece of ice is still visible (approximately one minute).
Note: Do not shake the vial during thawing.

3.3 Bring the vial to the biological cabinet immediately and spray the outside of the vial thoroughly with 70% ethanol and wipe it with an autoclaved paper towel.
3.4 Remove the cells from the vial using a p1000 micropipette (or serological pipette) and transfer it slowly, drop-wise while swirling into a 15 mL conical tube containing 5 mL of pre-warmed NK Cell Culture Medium. Wash the vial with 1 mL medium from the 15 mL conical tube and transfer it back to the tube.
Note: Do not mix cells up and down and avoid generation of bubbles.

3.5 Centrifuge cells at 250 x g for 5 minutes at room temperature.
3.6 Aspirate the medium very carefully using a vacuum (or pipette if preferred), leaving only a drop of liquid in the tube.
Note: Take extra care not to remove or disturb the cell pellet during aspiration of medium.

3.7 Using a p1000 micropipette, add 1 mL of the pre-warmed NK Cell Culture Medium into the tube and gently re-suspend cells by pipetting up and down 2-3 times.
3.8 Remove a 10 µL aliquot of the cell suspension and mix it with 10 µL of Trypan blue solution.
3.9 Count the cells.
3.10 Seed the NK cells at a density ranging from 50,000 – 100,000 live cells/cm^2 in NK Cell Culture Medium. Distribute the cells evenly.
3.11 Place cell culture vessels in the incubator (37°C/ 5% CO_2/ humidity control) overnight.
3.12 Half change media every other day.
3.13 Once the cells are confluent, split the cells by seeding at 50,000-100,000 live cells/cm^2.