Applied StemCell, Inc.
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Datasheet

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

Product Information

Catalog Number **ASE-9720**

Description

Applied StemCell has developed an efficient integration-free, small molecule-based method to differentiate high-quality T cells from human iPSCs. The differentiated T cells recapitulate the phenotype and functional parameters of primary and *in vivo* T cells.

We provide T cells differentiated from an integration-free, control human iPSC line (ASE-9211), reprogrammed from the fibroblasts of an African-American male donor. These cells express T cell biomarkers, CD4+ and CD8+ (Figure 1).

To harness the full potential of our T cells, we also provide optimized T Cell Basal Media (ASE-9720MM) and T Cell Supplement (ASE-9720MM-A) that support robust maintenance and functionality of the T cells in culture.

These iPSC-differentiated T cells can be used as control lines to compare phenotype and functionality of patient-derived, genome edited iPSC-derived T cells for drug screening applications. These T cells can also be used as effector cells for cytotoxicity assays.

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 T cells has been tested for growth, viability and purity following recovery from cryopreservation. In addition, each lot has been tested for expression of T cell markers, 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

The performance of Applied StemCell's iPSC-derived T cells has been validated with the T Cell Basal Media (ASE-9720MM) and T Cell Supplement (ASE-9720MM-A) provided in the T Cell Kit and the recommended additional reagents. Applied StemCell will not hold responsibility if components other than the components provided with the T Cell Kit and those recommended are used to culture the Applied StemCell T 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-9720 T Cells

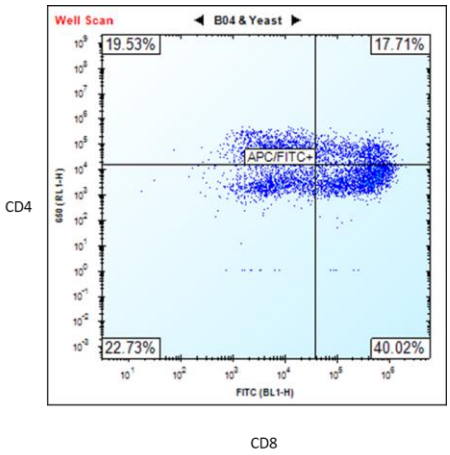

Figure 1. **Flow cytometry analysis of ASE-9720 iPSC-derived T cells for T cell biomarkers.** The T cells, differentiated from Applied StemCell's control iPSC line, ASE-9211, were stained with T cell markers CD4 and CD8. (Premature T Cells CD4+/CD8+ = 17.71% and Mature T Cells CD8+ = 40.02%) *

**Upon request, the T cells can be purified by FACS or MACS (single positive or double positive). We can also further differentiate the cells to obtain a higher percentage (≥80) of mature CD8+ T cells.*

Media and Materials

T Cell Kit (ASE-9720)

Catalog #	Component	Amount	Storage	Shelf Life
ASE-9720-C	iPSC-derived T Cells (CD4+, CD8+); African-American Male Line	≥1x10^6 cells/ vial	Liq. N2	12 months
ASE-9720MM	T Cell Basal Media	100 mL	-20°C	12 months
ASE-9720MM-A	T Cell Supplement	1 mL	-20°C	12 months

Additional Reagents Required

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

- Conjugated antibodies:
  - CD4 Capricobio #112044
  - CD8 Capricobio #100614

Protocol

1. Preparation of T Cell Culture Media

1.1. Thaw the T Cell Basal Media and Supplement at room temperature before thawing the cryopreserved T cells.
1.2. Add Supplement into Basal Media and mix well to make T Cell Culture Media.
1.3. The Culture Media should be aliquoted and stored at -20°C if it will not be used immediately.
Note: The 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 T Cells

2.1 To thaw the cryopreserved T cells, remove one vial from the storage unit.
2.2 Immerse the vial in the water 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.

2.3 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.
2.4 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 T 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 generating bubbles.

2.5 Centrifuge cells at 250 x g for 5 minutes at room temperature.
2.6 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.

2.7 Using a p1000 micropipette, add 1 mL of the pre-warmed T cell culture medium into the tube and gently re-suspend cells by pipetting up and down 2-3 times.
2.8 Remove a 10 µL aliquot of the cell suspension and mix it with 10 µL of Trypan blue solution.
2.9 Count the cells.
2.10 Seed the T cells at a density ranging from 150,000-200,000 live cells/cm^2 in T Cell Culture Media.
2.11 Distribute the cells evenly.
2.12 Place the cell culture vessels in the incubator (37°C/ 5% CO_2/ humidity control) overnight.
2.13 We recommend changing half of the media every 3-4 days.