AKADEUM LIFE SCIENCES

Human T Cell Selection, Activation, and Expansion Kit - CR

REF

Catalog Number
13310-224CR800
Document Number
UG13310224CR800A01

Kit Contents:

QTY

8 mL BACS™ Selection, Activation, and Expansion Microbubbles - CR in storage buffer

0.04 mL Human T Cell Selection, Activation, and Expansion Antibody Cocktail - CR in sterile PBS

Expiration dates are indicated on the labels for each individual component.

Storage

This product is shipped refrigerated and must be stored between +2 °C and +8 °C immediately upon receipt. Do not freeze.

Product Description

The Human T Cell Selection, Activation, and Expansion Kit - CR (Clinical Ready) was developed with BACS™ Microbubbles to isolate, activate, and expand T cells from peripheral blood mononuclear cell (PBMC) populations in one simple system.

Intentions for Use

This kit is designed to isolate, activate, and expand T cells from up to 800 x 10^6 starting PBMCs.

The components of the Human T Cell Selection, Activation, and Expansion Kit – CR are intended for the ***ex vivo*** isolation of human T cells from PBMCs for cell-based clinical research. They are not intended for human ***in vivo*** use.

Quality Statement

GMP Clinical Ready

Akadeum CR products are manufactured according to cGMP at Akadeum Life Sciences, Ann Arbor, MI, under a quality management system in compliance with 21 CFR 820, 211, and 210. They are developed following USP <1043> recommendations on ancillary materials and tested according to ISO 20399 and ISO 10993.

All components tested for endotoxins as per USP <85> Bacterial Endotoxins.

STERILE A

Sterile as per USP <71> Sterility Tests. All components manufactured in conjunction with in-process electron beam processing and filled aseptically.

Safety Information

For information regarding hazards and safe handling practices, please consult the Safety Data Sheet.

Animal Origin

The kit and its antibodies are produced under virus- and serum-free conditions.

Warnings

Do not use after the use-by date listed on the product label.

Do not use product if package is damaged.

Handling Guidelines

- When working with human blood products, including; cells, serum, and plasma, follow universal precautions.
- Proper Personal Protective Equipment (PPE) including lab coats, gloves, and eye protection are recommended when working with human tissues.
- Open solution transfers like spike connections and pipetting must be performed under a class 100 biological safety cabinet using aseptic technique.
- Human blood products must be treated as a potential source of HIV, HBV, and other bloodborne pathogens.
- Materials contaminated with blood products should be disposed of in labeled biohazard containers or decontaminated by site approved decontamination methods.

Additional Supplies Required

- 20 rpm end-over-end tube rotator for mixing
- Centrifuge (swinging bucket rotor strongly recommended)
- Vacuum aspirator
- Sterile 5 mL tubes / 50 mL tubes
- Buffer of choice
- Cytokine supplements as needed

Limited Warranty

Akadeum Life Sciences warrants their products as set forth in their General Terms and Conditions. Questions and requests can be sent to info@akadeum.com.

Related Products

Catalog Number	Product
13310-224GMP800	Human T Cell Selection, Activation, and Expansion Kit - GMP
13210-221GMP	Human T Cell Leukopak Isolation Kit - GMP
13510-232CR	Human T Cell Depletion Kit - CR (5 Billion Cells)
13510-232GMP	Human T Cell Depletion Kit - GMP (5 Billion Cells)

Before You Begin

- This user guide has been written for PBMCs or pre-isolated T cells. For alternative starting materials, please contact techsupport@akadeum.com.
- For maintenance of sterility, cell isolation should be conducted in a biosafety cabinet using aseptic technique.

Instructions for Use

Isolation of T cells with microbubbles using positive selection

1. Resuspend PBMCs or pre-isolated T cells in desired culture media (without cytokines) at approximately 3.3 x 10^8 cells / mL.

   Note: Both serum-free and serum-containing media formulations are acceptable; the addition of serum may improve T cell capture as well as increase expansion kinetics. For alternative starting materials, such as platelet washed apheresis material, or for in-bag closed system workflows contact techsupport@akadeum.com .

2. Add 5 µL selection, activation, and expansion antibody cocktail for every 1 x 10^8 total cells. Gently mix, and incubate at room temperature for 10 min.

3. Resuspend selection, activation, and expansion microbubbles by rapidly rolling the vial several times between hands followed by inverting multiple times to create a homogenous suspension. Ensure microbubbles remain as a homogenous suspension immediately prior to addition to sample. Add 1 mL microbubbles for every 1 x 10^8 total cells.

   Note: Add additional media, if needed, such that approximately 50-80% of the total vessel volume should be filled with media.

4. Mix using a commercial end-over-end (EOE) rotator at 20 rpm for 10 min at room temperature.

5. Separate microbubble-bound cells from undesired cells: Centrifuge for 5 min at 200 g at room temperature; use of a swinging-bucket rotor is strongly encouraged.

6. After centrifugation, the positively selected cells will be at the top of the suspension with the selection, activation, and expansion microbubbles. The remaining non-selected cells will be in a cell pellet or in suspension at the bottom of the vessel. Carefully retrieve the sample with minimal disturbance of the microbubble-cell layer. Using a sterile 9" glass pipet, insert the tip below the microbubble-cell layer and slowly descend to the bottom of the tube, manually aspirate the cell pellet and subnatant with an electronic pipette and transfer them to a new tube. Be careful not to aspirate the floating microbubble-bound cells.

   Note: Retaining the subnatant and unwanted cells will allow for indirect quantification of T cell capture efficiency.

Quantification of the positively selected T cells

7. Resuspend the bubble-bound cells in 1 mL of desired culture media and set it aside.

8. Count the cells in the subnatant using an automated cell counter or preferred cell counting technique and subtract this value from the starting cell number to determine the number of T cells captured in the bubble-cell layer.

9. Resuspend the microbubble-bound cells remaining in the original vessel in complete T cell medium (or other desired medium with preferred cytokine supplementation). Add approximately 1 mL media for every 0.5 x 10^6 T cells recovered. Akadeum recommends adding cytokine supplements for T cell culture, such as 50 U/mL IL-2, 5 ng/mL IL-7 and 5 ng/mL IL-15. Cytokine concentrations should be optimized for your particular needs.

Seeding of T cells in cell culture medium

10. Distribute cells at a concentration of 0.5 x 10^6 T cells / mL in a desired cell culture vessel for incubation.

Ongoing expansion of T cells

11. Monitor cell growth and refresh media and cytokines every 2-3 days as needed. For well plate studies, remove half of the media from the midnatant and replace with fresh media and cytokines. For bag culture studies, add fresh media with cytokines.

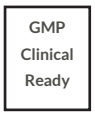
12. When the cell density exceeds 2 x 10^6 -2.5 x 10^6 cells/mL, transfer the cells into a larger vessel and/or dilute them to 0.5 x 10^6 cells/mL to allow for further expansion.

    Note: Removal of the selection, activation, and expansion microbubbles from culture is not required. However, microbubbles may be removed from the culture 2 days after transduction (or a minimum of 4 days in culture).

Glossary of Symbols:

Catalog Number

Contents of packaging



Manufactured using Good Manufacturing Practices

Sterilized using aseptic processing techniques


Temperature Limit


Use-by date


Do not use if package is damaged

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Patent No. 11,291,931
Visit www.akadeum.com for how-to videos, additional product information, and tech support

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