

For research use only

INTRODUCTION

CD4 is an accessory molecule. This antigen binds to MHC class II molecules and is a co-receptor in MHC class II restricted antigen-induced activation. CD4 is expressed on T helper cells and at a lower level on thymocytes and monocytes. The CD4 molecule is a receptor for the HIV-1 envelope protein gp120 and plays an important role in the aggressive infection process.

PRODUCT DESCRIPTION

Cell-Adembeads are superparamagnetic nanoparticles optimized for the positive selection and depletion of cells using commercially available separation columns.

Cell-Adembeads are designed for direct labeling of cells. We have developed a specific polymer on the surface of the beads and selected highly specific antibodies, thus reducing non specific capture and optimizing the separation.

The small size combined to the specific polymer allows the cells to be further evaluated in downstream application like flow cytometry or functional studies such as proliferation or cytotoxicity assay.

PRODUCT PRINCIPLE

CD4+ cells are labeled with CD4 Cell-Adembeads. Magnetic labeled cells are separated from unlabeled cells by passing them through a separation column that has been placed in a magnetic field.

For isolation of highly pure T helper cells, we recommend combining depletion of monocytes with CD14 Cell-Adembeads and positive selection with CD4 Cell-Adembeads.

COMPONENT DESCRIPTION

The Kit contains all of the components required for 10^9 total cells separation. CD4 Cell-Adembeads are uniform sized superparamagnetic nanoparticles conjugated to monoclonal mouse anti-human CD4 antibodies. Cell-Adembeads are produced and supplied in an aqueous suspension containing a preservative 0.05% Proclin 300. Dilution Buffer is produced under aseptic conditions. Content is sterile in unopened tube.

	Amount	Component	Storage
R1	125µl	CD4 Cell-Adembeads	+ 2-8°C
R2	15 ml	Dilution Buffer	+ 2-8°C

Table 1: Components provided with the kit

INSTRUCTION FOR USE

A) Before starting

To prevent cell death and non specific cell labeling, we recommend working rapidly and performing cell separation on ice in addition to using pre-cooled solutions.

B) Sample preparation

1. Prepare PBMCs suspension by density gradient centrifugation from anticoagulated peripheral blood or buffy coat.
2. At the end of PBMCs preparation, **resuspend cell pellet at a concentration of 10^8 cells/ml in cold Dilution Buffer.**

Notes:

- For frozen mononuclear cells, we recommend incubating cells with 100µg/ml DNase for at least 15 minutes at room temperature prior to labeling and separation.
- To remove cells clumps and avoid blocking the separation column, pass cells through a 30 µm nylon mesh.
- To remove platelets after density gradient separation, resuspend cell pellet in PBS and centrifuge at 200g for 10–15 minutes at 20 °C. Carefully remove supernatant. Repeat washing step and carefully remove supernatant.
- For flow cytometry analyses of initial samples, dilute cell suspension in PBS 0.5% BSA 2mM EDTA pH 7.5 buffer after staining with fluorescent antibodies.

C) CD4 Cell-Adembeads preparation

For optimal performance, match the amount of Cell-Adembeads to the amount of the total cells.

We recommend using 12.5µl diluted Cell-Adembeads per 100µl of the cell suspension at 10^5 cells / ml

3. Dilute CD4 Cell-Adembeads 10-fold with Dilution Buffer.

Notes:

- For optimal performance, the use of Dilution buffer is critical and the mixture (beads in dilution buffer) must be prepared just before the magnetic labeling.

D) Magnetic labeling

4. **Add the cell suspension to the diluted CD4 Cell-Adembeads.** Homogenize by pipetting and **incubate for 15 min at 4-8°C.**
5. (Optional for assessing purity) Add staining antibody and incubate for 10 min at 4-8°C.
6. **Add 500µl of PBS 0.5% BSA 2mM EDTA pH 7.5** for a total cell number up to 10^8 cells.

Do not centrifuge cell / bead complexes

7. **Cells are ready for separation.** Follow directions recommended by column manufacturer.

Notes:

- Step D6: for higher cell number, scale up buffer (PBS 0.5% BSA 2mM EDTA pH 7.5) volume accordingly.
- EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD).
- BSA can be replaced by other proteins such as human serum albumin, human serum or fetal calf serum.
- Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.

TROUBLESHOOTING

1. The purity is too low:

- Decrease the volume of Cell-Adembeads

- Check, you have used Dilution Buffer.

2. The recovery is too low:

- Increase the volume of Cell-Adembeads.
- Incubate preferentially at room temperature, 15 minutes cell / bead complexes by mixing (1000 rpm).

STORAGE / STABILITY

When stored in unopened vials at 2-8°C, Cell-Adembeads are stable until expiration date printed on the label.

The Cell-Adembeads must be maintained in liquid during storage and all handling steps. Drying will result in reduced performance. Do not freeze the product.

PRECAUTIONS

Precautions should be taken to prevent bacterial contamination of protein-coated Adembeads.

If cytotoxic preservatives are added they must be carefully removed before use by washing.

WARNINGS AND LIMITATIONS

For in vitro research only. Not for use in human diagnostic or therapeutic procedures.

Avoid pipetting by mouth.

WARRANTY

The products are warranted to the original purchaser only to conform to the quality and contents stated on the vial and outer labels for duration of the stated shelf life.

Ademtech's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Ademtech's expense, of any products which shall be defective in manufacture, and which shall be returned to Ademtech, transportation prepaid, or at Ademtech's option, refund of the purchase price.

Claims for merchandise damaged in transit must be submitted to the carrier.

Ademtech SA - Parc scientifique Unitec 1 - 4, allée du Doyen G. Brus - 33600 PESSAC – FRANCE

www.ademtech.com