

## Human CD4+ Cell Separation Kit (RUO)

### 1 Product Information

Product Name	Cat No.	Components
Human CD4+ Cell Separation Kit (RUO)	K1202-10	1 mL anti-human CD4 biotin antibody 1 mL Streptavidin MicroBeads

### 2 Product description

**This product is for research use only.**

Human CD4+ Cell Separation Kit is used for quick and easy separation of CD4+ cells from single-cell suspensions of human peripheral blood mononuclear cells (PBMC), thymus, lymph nodes or other tissues.

**Principle:** The labeled CD4+ cells are obtained by adding appropriate amount of antibodies and MicroBeads into the single-cell suspension and by magnetic adsorption on the columns. The separated cells can be directly used for downstream experiments such as cell culture and flow cytometry etc.

### 3 Capacity

For  $10^9$  total cells, up to 100 tests ( $10^7$  cells/test).

### 4 Transportation and storage

Shipping at  $2 \sim 8^\circ\text{C}$ ;

Store protected from light at  $2 \sim 8^\circ\text{C}$ . Do not freeze. Valid for 12 months.

### 5 Requirements for reagents and instruments

Buffer: phosphate buffered saline (PBS) pH 7.2, containing 0.5% bovine serum albumin (BSA) and 2 mM EDTA;

LarSep Columns (RWD, Cat No. HCSC-25);

30  $\mu\text{m}$  cell filter.

#### Note:

- PBS containing  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  is not recommended.
- To prevent air bubbles from blocking the column, please avoid using buffers that contain many bubbles.

### 6 Method for use

#### 6.1 Sample preparation

- 1) For human peripheral blood, it is suggested to use density gradient centrifugation to obtain peripheral blood mononuclear cells (PBMC); for other tissues, prepare single-cell suspension with the Single cell suspension dissociator or manually.
- 2) Rinse the 30  $\mu\text{m}$  cell filter with buffers, and then filter the cell suspension with the filter. After preparation, store the cell suspension at  $2 \sim 8^\circ\text{C}$ .
- 3) (Optional) Dead cells or erythrocytes may affect the separation, which can be depleted by Dead Cell Removal Kit and Red Blood Cell Lysis Buffer.

#### 6.2 Magnetic labeling

##### Note:

- The reagents given in the following steps can process  $10^7$  cells. If there are less than  $10^7$  cells, add the reagents according to  $10^7$  cells; if there are more than  $10^7$  cells, increase the reagents accordingly in proportion.
- Operate as quickly as possible, keep cells cold, and use pre-cooled solutions to reduce nonspecific cell labeling.

- 1) Count cell number and adjust the cell concentration to  $1 \times 10^8$  cells /mL.
- 2) Take 100 $\mu\text{L}$  cell suspension (containing  $10^7$  cells).
- 3) Add 10 $\mu\text{L}$  antibody.
- 4) Mix well and incubate at  $2 \sim 8^\circ\text{C}$  for 10 min.
- 5) Wash cells with 1 ~ 2 mL buffer, centrifuge at  $500 \times g$  for 5 min and discard the supernatant.
- 6) Resuspend cells with 100 $\mu\text{L}$  buffer.
- 7) Add 10 $\mu\text{L}$  Streptavidin MicroBeads.
- 8) Mix well and incubate at  $2 \sim 8^\circ\text{C}$  for 15 min.
- 9) Wash cells with 1 ~ 2 mL buffer, centrifuge at  $500 \times g$  for 5 min and discard the supernatant.
- 10) Resuspend cells with 500  $\mu\text{L}$  ~ 1 mL buffer if there are no more than  $1.25 \times 10^8$  cells. Increase buffer if there are more cells.
- 11) Perform magnetic separation. (Filter sample before separation if cell concentration is too high or there are too much cell clumps and aggregates)

### 6.3 Magnetic separation

**Note:** Before adding buffer in the following steps, make sure that all the buffer added to the column in the previous step drains away (i.e. no continuous droplets are dripping from the lower port of the column).

- 1) Put the column in a suitable magnetic field.
- 2) Wash the column with 2 mL buffer.
- 3) Add the cell suspension to the column and collect the effluent (containing unlabeled cells).
- 4) Wash the column with 2 ~ 3 mL buffer and collect the effluent, and mix it with the effluent collected in step 3). Repeat washing for 2 ~ 3 times.
- 5) When buffer added in the previous step drains away, remove the column from the magnetic field and replace the collection tube with a new one.
- 6) Add 1 ~ 2 mL buffer into the column and then flush out the buffer with the plunger supplied with the column to obtain the magnetic labeled cells.
- 7) (Optional) To improve purity of the magnetic labeled cells, repeat steps 3) to 6) to enrich the magnetic labeled cells.

### 7 Precautions

- 1) This product is for research use only, not for diagnostic or therapeutic use.
- 2) This kit is valid for 12 months, and RWD does not guarantee the validity of expired products.
- 3) All operations should be performed under sterilized conditions.
- 4) Cells should be incubated at 2 ~ 8°C. High temperatures or extended incubation duration may result in non-specific labeling.

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