

## Streptavidin Nano-Magnetic beads (RUO)

### 1 Product Information

Product Name	Model	Components
Streptavidin Nano-Magnetic beads (RUO, Trial)	K1200-10T	200 $\mu$ L Streptavidin Nano-Beads
Streptavidin Nano-Magnetic beads (RUO)	K1200-10	1 mL Streptavidin Nano-Beads

### 2 Product Description

Streptavidin MicroBeads are developed for the separation of cells according to surface markers labeled with biotinylated antibodies or ligands. The product has small particle size, good biocompatibility and no irritation to cells.

Principle: The labeled 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  $1 \times 10^9$  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, Model. HCSC-25)

40  $\mu$ 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 40  $\mu$ m cell filter with buffers, and then filter the cell suspension with the filter. After preparation, store the cell suspension at  $2 \sim 8^\circ\text{C}$ .
- (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) Determine cell number.
  - (2) Centrifuge cell suspension at  $500 \times g$  for 5 minutes. Aspirate supernatant completely.
  - (3) Resuspend the cells with an appropriate volume of buffer (the resuspension volume is related to the concentration of the antibody added for subsequent incubation).
  - (4) Add the amount of antibody recommended by the manufacturer, or do an antibody titer test in advance

to select the most applicable amount (100  $\mu$ L system incubation of antibody is recommended for  $10^7$  cells).

- (5) Mix well and incubate at 2 ~ 8°C for 10 min.
- (6) Wash cells with 1 ~ 2 mL buffer, centrifuge at 4°C, 500 $\times$ g for 5 min and discard the supernatant.
- (7) Resuspend cells with 90  $\mu$ L buffer.
- (8) Add 10  $\mu$ L **Streptavidin Nano-Beads**.
- (9) Mix well and incubate at 2 ~ 8°C for 15 min.
- (10) Wash cells with 1 ~ 2 mL buffer, centrifuge at 4°C, 500 $\times$ g for 5 min and discard the supernatant.
- (11) Resuspend cells with 500  $\mu$ L ~ 1 mL buffer if there are no more than  $1.25 \times 10^8$  cells. Increase buffer if there are more cells.
- (12) Perform magnetic separation (Filter sample before separation if cell concentration is too high or there are too many 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 4 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 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 Precautions

- (1) This product is for research use only.
- (2) All operations should be performed under sterilized conditions.
- (3) Do not mix and match components from different lots or different kits.
- (4) This kit is valid for 12 months, and RWD does not guarantee the validity of expired products.
- (5) Cells should be incubated at 2 ~ 8°C. High temperatures or extended incubation duration may result in non-specific labeling.
- (6) When the percentage of target cells is low or high, the amount of magnetic beads can be appropriately reduced or increased to obtain higher purity.

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