CD11b+ (Microglia) Cell Separation Kit Instruction

1 Product Information

Product Name	Model	Components
CD11b+ (Microglia) Cell Separation Kit	K1306-10	1 mL anti-mouse CD11b biotin antibody 1 mL Streptavidin MicroBeads
CD11b+ (Microglia) Cell Separation Kit (Trial)	K1306-10T	200 μL anti-mouse CD11b biotin antibody 200 μL Streptavidin MicroBeads

2 Description

The CD11b+ (Microglia) Cell Separation Kit enables separation and purification from single-cell suspensions of human or mouse tissues such as brain (tumor) to obtain microglia. The product has small particle size and good biocompatibility, which does not affect the subsequent experiments.

Main principle: Obtain the target microglia by adding appropriate amounts of MicroBeads to single-cell suspensions and by magnetic adsorption and using the LarSep Columns. The obtained microglia can be used directly for analysis of nucleic acids, proteins and cell functions or further cultured for other downstream experiments.

3 Capacity

For $1*10^9$ total cells, up to 100 tests (10^7 cells/test).

4 Transport and Storage

Shipping at 2 ~ 8°C;

Store protected from light at 2 ~ 8°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 μm cell filter

Note:

- PBS containing Ca^{2+} or 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) When processing mouse embryo/young mouse brain and adult mouse brain (including spinal cord), prepare the single-cell suspension using the Single Cell Suspension Dissociator or manually.

(2) (Optional) Dead cells or erythrocytes may affect the separation, which can be depleted by Dead Cell Removal Kit and Red Blood Cell Lysis Buffer.

(3) When processing viscous samples such as young mouse brain (≤ P7), it is feasible to filter the single-cell suspension with a filter mesh with a pore size of 40 μm before separation and add buffer to 2 ~ 3 mL of resuspended cells in Step (10) described in the reaction of 6.2. Store the cell suspension at 2 ~ 8℃ upon completion of the preparation for future use.

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 1×10^8 cells /mL.
(2) Take 100 μL cell suspension (containing 10^7 cells).
(3) Add 10 μL anti-mouse CD11b biotin antibody.
(4) Mix well and incubate at 2 ~ 8 ℃ for 10 min.
(5) Wash cells with 1 ~ 2 mL buffer, centrifuge at 500×g for 5 min and discard the supernatant.
(6) Resuspend cells with 100 μL buffer.
(7) Add 10 μL Streptavidin MicroBeads.
(8) Mix well and incubate at 2 ~ 8°C for 15 min.
(9) Wash cells with 1 ~ 2 mL buffer, centrifuge at 500×g for 5 min and discard the supernatant.
(10) Resuspend cells with 500 μL ~ 1 mL buffer if there are no more than 1.25×10^8 cells. Increase buffer if there are more cells.
(11) Performing magnetic labeling.

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 LarSep Columns.
(4) Collect the effluent, wash the LarSep Columns with 2 mL of buffer for 2 ~ 3 times, and collect the effluent (containing unlabeled cells).
(5) After the buffer added in the previous step flows out, move the LarSep Columns from the magnetic field to a new collection tube, add 2 mL of buffer, and push out the buffer using the matching piston of the LarSep Columns to obtain the labeled target cells.

7 Precautions

(1) Do not mix and match components from different lots or different kits.
(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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