

Pan T Cell Isolation Kit ,human (92-01-0262)

[Components]

1 mL Pan T Cell Biotin-Antibody Cocktail, human: Cocktail of biotin-conjugated monoclonal antibodies against CD14, CD15, CD16, CD19, CD34, CD36, CD56, CD123, and CD235a (GlycophorinA).

2 mL Pan T Cell MicroBead Cocktail, human: MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1) and monoclonal anti-CD61 antibody (isotype: mouse IgG1).

[Capacity] For 10^9 total cells.

[Product format] All components are supplied in buffer containing stabilizer and 0.05% sodium azide.

[Storage] Store protected from light at 2 – 8 °C. Do not freeze. The expiration date is indicated on the vial labels.

[Cell separation protocols]

1. Fully automated cell labeling and separation using the Pro Separator
2. Manual magnetic labeling

[Reagent and instrument requirements]

● Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA. Degas buffer before use, as air bubbles could block the column.

- (Optional) Pre-Separation Filters (30 μm) to remove cell clumps.
- Choose the appropriate Separator and Columns:
- ▲ **Note:** When using this kit the unwanted cell fraction is labeled and the target cells remain unlabeled. Depending on the target cell frequency, the labeled fraction can therefore represent the majority of the total cells.

To avoid blocking of the column, do not exceed the max number of labeled cells per column. Estimate the number of labeled cells in the sample, split the sample if necessary and use the appropriate number of separation columns.

[Fully automated cell labeling and separation using the Pro Separator]

- ▲ Refer to the user manual for instructions on how to use the Pro Separator.
- ▲ All buffer temperatures should be ≥ 10 °C.
- ▲ Place tubes in the following Chill Rack positions:
position A = sample, position B = negative fraction, position C = positive fraction

1. Switch on the instrument for automatic initialization.
2. Go to the **Reagent** menu and select **Read Reagent**. Scan the 2D barcode of each reagent vial with the barcode scanner on the Pro Separator. Place the reagent into the appropriate position on the reagent rack.
3. Place sample and collection tubes into the Chill Rack.
4. Go to the **Separation** menu and select the reagent name for each sample from the **Labeling** submenu (the correct labeling, separation, and wash protocols will be selected automatically).

5. Enter sample volume into the **Volume** submenu. Press **Enter**.
6. Select **Run**.
7. Collect enriched T cell fraction at position B = negative fraction.

[Subsequent automated cell separation using the Pro Separator]

▲ Refer to the user manual for instructions on how to use the Pro Separator.

▲ All buffer temperatures should be ≥ 10 °C.

▲ Place tubes in the following Chill Rack positions:

position A = sample, **position B** = negative fraction, **position C** = positive fraction

8. Prepare and prime the instrument.
9. Follow the instructions that are given in the user manual.
10. The program “Depletes” is recommended. Collect enriched T cells at position B = negative fraction.

[Manual magnetic labeling]

▲ Work fast, keep cells cold, and use pre-cooled solutions (2–8 °C).

▲ Volumes for magnetic labeling given below are for up to 10^7 total cells. When working with fewer cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling.

1. Prepare cells and determine cell number.
2. Resuspend cell pellet in 40 μL of buffer per 10^7 total cells.
3. Add 10 μL of Pan T Cell Biotin-Antibody Cocktail per 10^7 total cells.
4. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
5. Add 30 μL of buffer per 10^7 total cells.
6. Add 20 μL of Pan T Cell MicroBead Cocktail per 10^7 total cells.
7. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
8. Proceed to subsequent magnetic cell separation.

▲ **Note:** A minimum of 500 μL is required for magnetic separation. If necessary, add buffer to the cell suspension.

[Subsequent manual cell separation]

▲ Always wait until the column reservoir is empty before proceeding to the next step.

9. Place LS column in the magnetic field of a suitable Separator.
10. Prepare column by rinsing with 3 mL of buffer.
11. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched T cells.
12. Wash column with 3 mL of buffer. Collect unlabeled cells that pass through, representing the enriched T cells, and combine with the effluent from step 11.

13. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette 5ml of buffer onto the column. Immediately flush out the magnetically labeled non-T cells by firmly pushing the plunger into the column.