Version：01 Update：12/30/2023

CytoSinct™ TCR αβ Nanobeads, human

Contents

I. Product Description

The CytoSinct™ TCR αβ Nanobeads, human is used for *in vitro* enrichment or depletion of TCRα/β+ cells from fresh or frozen peripheral blood mononuclear cells (PBMCs), leukapheresis products or single cell suspension based on the surface expression of human TCRα/β. The surface of Nanobeads are labeled with an anti-human TCRα/β monoclonal antibody.

To begin the isolation, Nanobeads are added to the cells.The cells/beads mixed suspension is loaded onto a CytoSinct Column which is placed in the CytoSinct Magnetic Rack, or other compatible columns with magnet.

TCRα/β+ cells labeled with CytoSinct Nanobeads are retained within the column and unlabelled cells are flushed out during the wash step. After removal of the magnetic field, TCRα/β+ cells can be easily eluted from the column as the positively selected cell fraction.

II. Product Specification

Cat. No.	Product Name	Size	Capacity
L00897-1	CytoSinct™ TCR αβ Nanobeads, human	1 mL	for up to 2.6×10^9 total cells
L00897-7.5	CytoSinct™ TCR αβ Nanobeads, human	7.5 mL	for up to 2×10^{10} total cells

Reactivity	Human
Product Format	Bio-degradable matrix coated nanoparticle conjugated to monoclonal mouse anti-human TCRα/β antibodies supplied in phosphate buffered-saline (PBS), containing Human Serum Albumin (HSA).
Storage	Store at 2-8 °C. Do not freeze.

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III. Required Materials

1. Isolation Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA) and 2 mM EDTA.

- Keep Isolation Buffer cold (2−8°C).
- BSA can be replaced by human serum albumin (HSA), human serum or fetal bovine serum (FBS).
- EDTA can be replaced by sodium citrate.
- **PBS containing Ca^{2+} or Mg^{2+} is not recommended.**

2. Columns and Separators:

- For samples containing less than 2×10^8 total mononuclear cells (MNCs) or less than 10^7 labeled cells, use CytoSinct gM Column and CytoSinct M1 or M8 Magnet, or other compatible columns and magnets.
- For samples containing less than 2×10^9 total cells or less than 10^8 labeled cells, use CytoSinct gL Column and CytoSinct L1 or L4 Magnet or other compatible columns and magnets.
- **For depletion, we suggest using gL columns regardless of starting cell numbers.**

IV. Protocol

All procedures are to be performed at room temperature unless otherwise instructed in this protocol. To achieve the capacity designated, an automated instrument is suggested, e.g. CytoSinct 1000. When using a manual approach, please refer to the protocol below.

1. Prepare Nanobeads

Gently mix the Nanobeads by pipetting for several times.

2. Prepare Samples

2.1 Prepare PBMCs.

- When working with anticoagulated peripheral blood, PBMCs should be isolated by density gradient centrifugation method and washed by Isolation Buffer to remove interfering factors.
- When working with frozen PBMCs, resuscitate frozen PBMCs and then proceed with the protocol. When dead cells are found to be considerable, apply density gradient centrifugation method (for example, using Ficoll-Paque™ PLUS density gradient media) to remove dead cells, or culture cells in medium overnight before proceeding with this protocol.

2.2 Centrifuge PBMCs at 300×g for 10 minutes at room temperature (15 - 25°C). Aspirate the supernatant completely. Determine cell number by using a hemocytometer or other suitable methods.

Note: for cell counting and viability determination, we recommend using **fluorescence-based**

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assays such as AO/PI instead of Trypan Blue. Trypan Blue tends to overestimate sample viability, particularly for blood samples.

3. Magnetic Labeling

3.1 Transfer desired number of cells into a new tube and resuspend into single cell suspension at 10^8 MNCs per 1 mL in Isolation Buffer.

- When working with less than 10^7 MNCs, use Isolation Buffer volume of 100 µL.

3.2 For TCRα/β+ cells enrichment, add **3.75-10 µL Nanobeads for each 100 µL cell suspension of 10^7 total MNCs** (e.g. for 2×10^7 total MNCs, use 7.5-20 µL Nanobeads).

- When working with less than 10^7 MNCs, use 3.75-10 µL Nanobeads as that in 10^7 MNCs (e.g. for 5×10^6 total MNCs, use 3.75-10 µL Nanobeads).

3.3 For **TCRα/β+ cells depletion**, an excessive amount of Nanobeads should be added, for example, **4-10 fold** of the amount used for enrichment.

3.4 Mix the Nanobeads and cells well by gently pipetting or tapping on the bottom of the tube, and incubate for 30 minutes at room temperature. Gently mix the sample every 3-5 minutes.

3.5 Wash cells once by adding 1-2 mL of Isolation Buffer per 10^7 MNCs, mix well by gentle pipetting, and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

3.6 Resuspend up to 10^8 cells in 500 µL of Isolation Buffer.

- Scale up the volume of Isolation Buffer accordingly when more than 10^8 MNCs are to be processed.

4. Magnetic Separation

4.1 Choose an appropriate CytoSinct Column and CytoSinct Magnet (or other compatible columns with magnet) according to the number of total cells and the number of TCRα/β+ cells as instructed in Section III.

4.2 Place the column onto the suitable magnet.

4.3 Rinse the column once with Isolation Buffer (500 µL for gM column, 3 mL for gL Column) and let the buffer run through it but not run dry.

4.4 Transfer the cell suspension onto the prepared gM or gL Column using a pipette and collect the unlabeled cells in flow-through.

4.5 Wash the column with Isolation Buffer (500 µL × 3 for gM Column, 3 mL × 3 for gL Column). Collect unlabeled cells in flow-through with a suitable tube (for example, a 2 mL or 15 mL conical tube). Repeat the washing step for another two times. Add new Isolation Buffer when the column stops dripping but not run dry.

4.6 Remove the column from the magnet and place it on a new tube with suitable size (for example, a 15 mL or 50 mL conical tube).

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4.7 Pipette the Isolation Buffer onto the column (1 mL for gM Column, 5 mL for gL Column). Immediately flush out the fraction with the magnetically labeled cells by firmly applying the plunger through the column chamber supplied with the column.

4.8 The cells can then be counted, analyzed to assess the purity or used in downstream applications. The Nanobeads do not need to be removed. To ensure cell viability, the desired cell fraction should be immediately resuspended in cell culture medium.

For research use only. Not intended for human and animal therapeutic or diagnostic use.