EXPERIMENTAL PROTOCOL: Isolation CD4+ T cells by immunomagnetic negative selection.

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Introduction

The intended use of this protocol is the characterization is to isolate highly purified CD4+ T cells directly from human whole blood by immunomagnetic negative selection, by using EasySep™ Direct Human CD4+ T Cell Isolation Kit.

Reagents

EasySep™ Direct Human CD4+ T Cell Isolation Kit (Ref. 19662)

1. EasySep™ Direct Human CD4+ T Cell Isolation Cocktail
2. EasySep™ Direct RapidSpheres™ 50300

- Phosphate Buffered Saline w/o Calcium w/o Magnesium (PBS)
- 1x RBC Lysis Buffer
- X-VIVO™ 20 Medium (Ref. BE04-448Q)
- Trypan Blue

Instruments and tools

- Laminar flow cabinet
- Centrifuge with swing-bucket rotors and acceleration and deceleration (braking) ramps
- Sodium Heparin Tubes
- 14 mL (17 x 95 mm) polystyrene round-bottom tube
- EASYSEP™ MAGNETS ‘’The Big Easy’’ (Catalog #18001)
- 96-well plate.
- Neubauer chamber

Before start

- RT - room temperature (15 - 25°C)

PROCEDURE

1. Collect sample within the volume range (1.5-7mL).
2. Add whole blood sample to 14mL polystyrene round-bottom tube.
3. Vortex RapidSpheres™ for 30 seconds.
4. Add 25 µl/ml Isolation Cocktail and 25 µl/ml RapidSpheres™ to sample.
5. Mix and incubate for 5 min at RT.
6. Add PBS to top up to double the volume for samples ≤ 5mL, or top up to 10mL for samples >5mL. Mix by gently pipetting up and down 2 3 times.
7. Place the tube (without the lid) into the magnet and incubate for 5 min at RT.
8. Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring the enriched cell suspension* into a new tube.
9. Add again 25 µl/ml RapidSpheres™ to a new tube containing enriched cells.
10. Mix and incubate for 5 min at RT.
11. Remove the tube from the magnet and place the tube from step 10 (without lid) into the magnet and incubate for a second separation for 5 min at RT.
12. Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring the enriched cell suspension into a new tube.
13. Remove the tube from the magnet and place the new tube from step 11 (without lid) into the magnet and incubate for a third separation for 5 min at RT.
14. Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring the enriched cell suspension into a new tube.
15. Add RBC Lysis Buffer to top up to 15 mL and incubate in rotation 10 min.
16. Centrifuge at 400g for 5 min at RT.
17. To minimize Red Blood Cells contamination in the isolated cells, repeat the step 15, 2 or 3 times.
18. Resuspend cells with 1mL of X-VIVO™ 20 Medium and proceed with cell counting.
19. Plate 100.000 cells/well in a 96 well plate with 200µL of X-VIVO™ 20.
20. Isolated cells are ready to use.