

ReadiPrep™ CD4+ Cell Isolation Kit
Optimized for processing 30 mL blood

 Catalog number: 67301
 Unit size: for 30 mL blood

Component	Storage	Amount (Cat No. 67301)
Component A: ReadiPrep™ CD4 Conjugate	Refrigerated (2-8 °C)	One vial (0.75mL)
Component B: Magnetic Spheres	Refrigerated (2-8 °C)	One vial (1 mL)
Component C: Isolation Buffer	Refrigerated (2-8 °C)	One bottle, ~25 mL
Component D: Release Buffer	Refrigerated (2-8 °C)	One bottle, ~30 mL

OVERVIEW

The ReadiPrep™ CD4+ Cell Isolation Kit provides a reliable and efficient method for isolating pure, viable CD4+ T cells from whole blood. This kit uses an immunomagnetic separation technique with specific CD4-conjugated beads, ensuring the targeted isolation of CD4+ cells while removing non-CD4+ cells and debris. The isolated CD4+ cells are then released from the magnetic beads.

The protocol is quick and straightforward, designed to be completed in under 30 minutes with minimal hands-on time. It is optimized for processing up to 30 mL of blood, with all necessary reagents (CD4 conjugate, magnetic spheres, isolation buffer, and release buffer) included in the kit. The isolated CD4+ cells are ideal for downstream applications, including flow cytometry, gene expression analysis, and immunological assays.

The ReadiPrep™ CD4+ Cell Isolation Kit offers high reproducibility and purity, making it an ideal choice for researchers studying immune responses, T cell activation, or conducting clinical trials.

AT A GLANCE
Protocol Summary (For Processing 1 mL Blood)

1. Take 1mL blood and incubate with 25uL ReadiPrep™ CD4 Conjugate (Component A).
2. Incubate for 10 min at RT.
3. Add 2mL of 1X isolation buffer and centrifuge at 350g for 15 min.
4. Remove the supernatant (remove the same volume of isolation buffer added).
5. Add 30uL Magnetic Spheres (Component B). Incubate 10 min at RT.
6. Place the tube on a magnetic rack.
7. Wait for 2-3 min. Remove supernatant (containing CD4 negative cells)
8. Wash beads with 1X Isolation Buffer.
9. Add Release Buffer to beads. Incubate for 10 min at RT (with rotating the tube)
10. Place the tube in a magnetic rack and collect the supernatant (containing CD4 positive cells).

SAMPLE EXPERIMENTAL PROTOCOL
For processing 1mL blood
Prepare 1x Isolation Buffer:

Add 10mL 10X Isolation Buffer (10X) (Component C) to 90mL cell culture water to prepare 1x Isolation buffer.

Blood Sample Preparation:

Take 1 mL whole blood cells with appropriate anticoagulants.

Stain with ReadiPrep™ CD4 Conjugate

Add 25 µL ReadiPrep™ CD4 Conjugate (Component A) to the 1 mL of blood sample.

Incubate for 10 min at RT.

Add 2 mL 1x Isolation Buffer and centrifuge tube at 350g for 15 min at RT.

Remove 2 mL supernatant. Keep 1 mL solution.

Isolate CD4+ Cells:

Add 30 µL Magnetic Spheres (Component B) to above 1 mL cells.

Incubate for 10 min at RT.

Place the tube on a magnetic rack.

Discard supernatant and wash the Magnetic Spheres (with CD4+ cells) with 1 mL of isolation buffer.

Repeat 2-3 times until you get a clear beads solution.

Release CD4+ Cells:

Add 500 µL Elution buffer (Component C).

Incubate 10 min at RT (rotating the tube).

Place the tube on a magnetic rack and collect supernatant containing CD4 positive cells.

To remove any residual beads, place the released CD4+ cells on a magnetic rack for one minute.

Transfer supernatant (CD4+ cells) to a new tube.

Note: This step can be performed twice to get more pure CD4+ positive cells.

The isolated cells are pure, viable, and are free of beads bound to the surface.

CD4+ Cells Storage

Add 500 µL 1x Isolation buffer to the isolated CD4+ cells and centrifuge at 300g for 10 min.

Remove supernatant and suspended cell pellets in any preferred medium.

Store at 4 °C.

Volume of reagents is scalable for specific volume of bloods:

WBC volume	Component A	Component B	Component C
1 mL	25 µL	30 µL	0.5-1 mL

EXAMPLE DATA ANALYSIS AND FIGURES

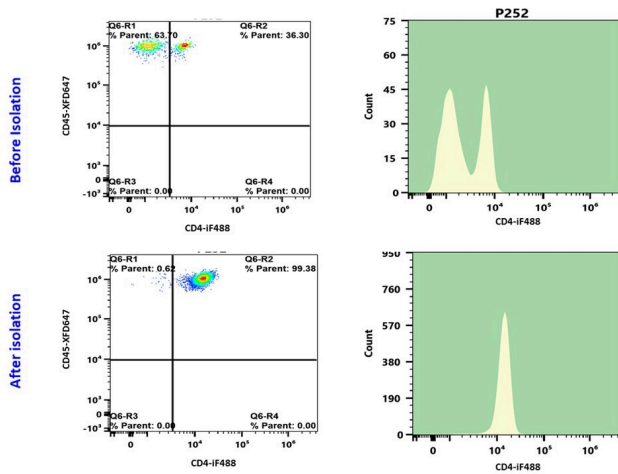


Figure 1. CD4+ T cells positively isolated from whole blood using ReadiPrep™ CD4+ Cell Isolation Kit. The figure shows that the percentage of CD4+ cells was 36.3% before isolation which increased to 99.38% after isolation. Cells were stained with CD4 (SK3)-iFluor® 488 and CD45 (2D1)-iFluor® 647.

DISCLAIMER

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