



CD15 MACSiBead™ Kit

human

Order no. 130-093-580

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1. Description

Components	2.5 mL CD15 MACSiBead™ Particles, human: MACSiBead™ Particles (appr. 3.5 µm diameter) conjugated to monoclonal anti-human CD15 antibodies (isotype: mouse IgM). 50 mL Red Blood Cell Lysis Solution (10x)
Capacity	For 50 mL human whole blood.
Product format	CD15 MACSiBead Particles are supplied in buffer containing 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of a separation using the CD15 MACSiBead™ Kit

First, the CD15⁺ cells are magnetically labeled with CD15 MACSiBead™ Particles. Then, the cell suspension is placed in the magnetic field of a MACSiMAG™ Separator. The bead-labeled cells migrate towards the magnet and adhere to the wall of the tube. The non-labeled cells in the supernatant are aspirated and collected as the non-labeled target cell fraction. After the depletion of CD15⁺ cells an erythrocyte lysis can be performed.

1.2 Background information

The CD15 MACSiBead Kit has been developed for the enrichment of lymphocytes from human whole blood by depletion of CD15⁺ cells, combined with the lysis of erythrocytes. CD15 MACSiBead Particles are superparamagnetic beads of approximately 3.5 µm in diameter, coupled to CD15 antibodies.

The CD15 antibody recognizes the carbohydrate structure 3-fucosyl-N-acetyl-lactosamine (3-FAL), also designated Lewis X or CD15 antigen. CD15 is expressed on neutrophils, eosinophils, and monoblastoid precursor cells of the myeloid lineage, but not on basophils and lymphocytes.

1.3 Applications

- Enrichment of untouched lymphocytes from human whole blood for downstream applications that tolerate erythrocyte lysis and do not require the isolation of pure lymphocyte subsets, e.g., cell enumeration, ELISA, or ELISpot analysis.
- Depletion of CD15⁺ cells from cryopreserved or stored blood samples prior to PBMC preparation by density gradient centrifugation.
- Depletion of CD15⁺ cells from fresh blood samples prior to cryopreservation.

1.4 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 by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
 - ▲ Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- MACSmix™ Tube Rotator (# 130-090-753) for incubation of cells with CD15 MACSiBead Particles.
- MACSiMAG Separator (# 130-092-168) for removal of cells labeled with CD15 MACSiBead Particles.
 - ▲ Note: Do not use MACSiBead Particles with MACS Columns and MiniMACS™, MidiMACS™, QuadroMACS™, OctoMACS™, VarioMACS™, SuperMACS™, or autoMACS™ Separators.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, for example, CD15-PE (# 130-091-375) and CD45-FITC (# 130-080-202). For more information about other fluorochrome conjugates see www.miltenyibiotec.com.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Preparation of whole blood

- ▲ Anticoagulants such as EDTA, heparin-EDTA, anticoagulant citrate dextrose solution formula A (ACD-A), or citrate phosphate dextrose (CPD) can be used. For subsequent molecular biology applications use EDTA as an anticoagulant.

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2.2 Magnetic labeling

▲ The CD15 MACSiBead Kit has been developed for the enrichment of lymphocytes by depletion of CD15⁺ cells from anticoagulated whole blood samples, ranging in volume from 0.5–40 mL.

▲ Resuspend CD15 MACSiBead Particles thoroughly before use, to obtain a homogenous dispersion of MACSiBead Particles in solution.

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for 0.5 mL of whole blood. When working with more than 0.5 mL of whole blood, scale up all reagent volumes and total volumes accordingly (e.g. for 1 mL of whole blood, use twice the volume of all indicated reagent volumes and total volumes).

▲ The recommended incubation temperature is 2–8 °C. Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Add 25 µL of CD15 MACSiBead Particles to 0.5 mL of whole blood.

▲ **Note:** (Optional) For immunofluorescent staining take an aliquot of the starting material before labeling with CD15 MACSiBead Particles.

2. Mix carefully and incubate for 15 minutes in the refrigerator (2–8 °C) using the MACSmix Tube Rotator (medium speed/ 8 rpm).

3. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Carefully resuspend cells. To avoid unintended detachment of MACSiBead Particles from magnetically labeled cells, do not vortex.

1. Place tube with cells labeled with CD15 MACSiBead Particles in the magnetic field of a MACSiMAG Separator.

▲ **Note:** Use tube rack to insert tubes from 1.5 mL to 5 mL in size. For details see the MACSiMAG Separator data sheet.

2. Allow the labeled cells to adhere to the wall of the tube:

0.5 mL, 1.5 mL, 2 mL, or 5 mL tubes: 2 minutes

15 mL or 50 mL tubes: 4 minutes

3. Retaining the tube in the MACSiMAG Separator, carefully pipette supernatant containing the non-labeled target cells into a new tube.

4. To remove residual MACSiBead Particles, place tube with non-labeled target cells in the MACSiMAG Separator and repeat steps 2 and 3.

5. Proceed to red blood cell lysis (2.4).

If red blood cell lysis is not desired, place tube in the MACSiMAG Separator and add 0.5 mL of buffer to the supernatant containing the non-labeled target cells. Repeat steps 2 and 3 to remove residual MACSiBead Particles.

2.4 (Optional) Red blood cell lysis

1. Prepare 5 mL of 1× Red Blood Cell Lysis Solution by adding 500 µL of Red Blood Cell Lysis Solution (10×) to 4.5 mL double-distilled water (dd H₂O).

▲ **Note:** Do not dilute with deionized water.

2. Add 5 mL of 1× Red Blood Cell Lysis Solution to 0.5 mL supernatant containing the non-labeled target cells.

3. Mix carefully and incubate for 10 minutes at room temperature.

4. Centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

5. Resuspend pellet in 0.5 mL of buffer.

6. To remove residual MACSiBead Particles, place tube with non-labeled target cells in the MACSiMAG Separator and allow the labeled cells to adhere to the wall of the tube:

0.5 mL, 1.5 mL, 2 mL, or 5 mL tubes: 2 minutes

15 mL or 50 mL tubes: 4 minutes

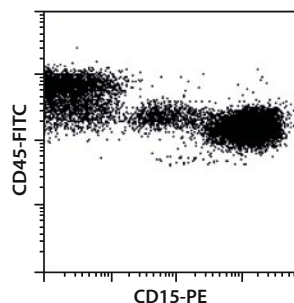
7. Retaining the tube in the MACSiMAG Separator, carefully pipette supernatant containing the non-labeled target cells into a new tube.

▲ **Note:** The non-labeled target cell fraction or an aliquot thereof can be stained with fluorochrome-conjugated CD45 and CD15 antibodies. For details, please refer to the respective fluorochrome data sheet.

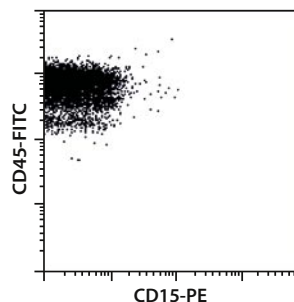
3. Example of a CD15⁺ cell depletion using the CD15 MACSiBead™ Kit

Enrichment of lymphocytes by depletion of CD15⁺ cells from human whole blood using the CD15 MACSiBead™ Kit and a MACSiMAG Separator, followed by treatment with Red Blood Cell Lysis Solution. Cells are fluorescently stained with CD15-PE and CD45-FITC and analyzed by flow cytometry. Gating was performed on CD45⁺ cells. Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.

Whole blood before separation



Enriched lymphocytes



All protocols and data sheets are available at www.miltenyibiotec.com.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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