# MagIso™ T Cell Isolation Kit

Cat.No: WHK-X001

## *DESCRIPTION*

**Description**

MagIso™ T Cell Isolation Kit (# WHK-X001) offers the user a quick method of separating T lymphocyte/Particles complexes from blood with the use of a collector magnet. The anti-CD2 monoclonal antibodies coupled to the particle surface specifically bind to the E-rosette receptor on T lymphocytes. The kit requires no cold incubations, rotations, or centrifugations and contains enough immunomagnetic particles to isolate more than 90% of the $CD2^+$ T cells in 1 mL of whole blood.

## *APPLICATION*

**Application Notes**

This kit provides a simple procedure for the isolation of T lymphocytes for use in microcytotoxicity assays using fluorescent dyes. MagIso™ Developer Buffer is a reagent specifically designed to enhance the performance of immunomagnetic particles.

## *KIT COMPONENTS*

**Kit Components**

| Kit Components | Storage |
|---|---|
| Magnetic Particles | 2-8 °C |
| Developer Buffer (10X) | 2-8 °C |

## *PRODUCT INFORMATION*

**Isolation Method** Positive Selection

**Sample Type** Blood

**Target Cell** T cells

## *MATERIALS*

**Materials Required But Not Supplied**

- Class I tissue typing trays
- 5 mL and 1.5 mL plastic or glass centrifuge tubes with caps
- Phosphate Buffered Saline (PBS) without $Ca^{++}$ and $Mg^{++}$ salts
- McCoy's medium or equivalent with 5% HIFCS
- Magnetic separator
- Aspirator or disposable pipettes
- Heat-Inactivated Fetal Calf Serum (HIFCS)

a) Stock solution: heat FCS at 56°C for 30 minutes to inactivate complement. Store at 2-5°C or aliquot and freeze at -20°C.

b) Working solution: add 5 mL HIFCS stock solution to 95 mL of McCoy's medium or equivalent.

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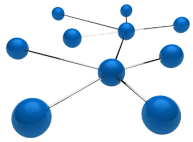


Optional Materials Not Provided

• Ficoll-Hypaque

• Percoll

a) Stock solution (Percoll-X): combine 1 part of 10X PBS and 9 parts of Percoll.

b) Working solution (50% Percoll): combine equal parts of Percoll-X and PBS.

• 1% Sodium Azide: Dissolve 1 gm sodium azide in 100 mL PBS.

• Carboxyfluorescein Diacetate (CFDA):

a) Stock CFDA solution: In a glass tube, dissolve 10 mg CFDA in 1 mL acetone. Store at -20°C. Store in dark.

b) Working solution: Use either of the following:

Prepared in PBS at pH 7.2: Add 30 µL stock CFDA solution to 5 mL PBS (pH 7.2). Store at 2-5°C for up to 1 week; Prepared in PBS at pH 5.5: Add 30 µL stock CFDA solution to 5 mL PBS (pH 5.5). Store at 2-5°C for up to 1 week.

## *EXPERIMENTAL PROCEDURE*

**Experimental Procedure**

Preparation

Magnetic particle: Resuspend before use by vortexing for 10 seconds.

MagIso™ Developer Buffer: Dilute one part (10X) stock solution to nine parts 1X PBS without $Ca^{++}$ and $Mg^{++}$ salts. Store at 2-5°C.

Sample: Draw approximately 10 mL of whole blood. The preferred anticoagulant is ACD or CPDA. Do not use lithium heparin! T cells should be isolated within 24 hours to achieve the highest yield. However, blood up to 3 days old can be used. Store blood specimen horizontally at room temperature (20-25°C).

Protocol 1. ISOLATION TECHNIQUES

A. Isolation from Whole Blood

1. Dispense 2 mL of blood into a 5 mL tube.

2. Resuspend magnetic particles thoroughly before use. Vortex approximately 10 seconds.

3. Add 100 µL magnetic particles to blood sample. Immediately cap tube and invert 2-3 times to disperse magnetic particles.

4. Rotate tube once per second for 3 minutes at 20-25°C to allow binding of particles to T cells. Do not exceed 4 minutes. Use an end-over-end rotating device or hand-mix.

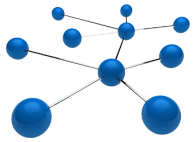
5. Add 2 mL of 1X Developer Buffer. Cap tube and invert 2-3 times to mix. This is an essential step!

6. Uncap and place tube in magnetic separator for a full 3 minutes.

7. Remove and discard supernatant with a disposable pipette. Remove tube from magnet.

8. Resuspend cells (particles) with 1-2 mL PBS. Gently flick tube to disperse particles. Replace tube in magnetic separator for 1 minute. Remove and discard supernatant. Repeat two times.

9. Proceed to the "Labeling and Cell Concentration Procedures" (below), or resuspend cells



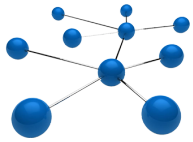
(particles) in 0.5 mL of McCoy's medium or equivalent with 5% HIFCS.

B. Isolation from Ficoll Interface

1. Centrifuge citrated or heparinized blood for 10 minutes at 400-900 g.
2. Collect buffy coat and dilute with an equal volume of PBS. Mix well.
3. Layer a maximum of 2 mL buffy coat/PBS mixture over 1.5 mL of Ficoll-Hypaque (Density (D) =1.077) in 5 mL tubes and centrifuge for 10 minutes at 1000 g.
4. Collect approximately 1 mL of interface from each tube and transfer into a centrifuge tube. Centrifuge for 1.5 minutes at 3000 g or 10 minutes at 1000 g.
5. Discard supernatant, resuspend pellet in PBS. Centrifuge for 5 minutes at 1000 g (removes the majority of platelets).
6. Discard supernatant with disposable pipette. Resuspend pellet in 1 mL of 20% HIFCS/PBS.
7. Dispense 100 µL of magnetic particles into sample tube and cap.
8. Rotate sample for 3 minutes at 20-25°C.
9. Uncap and place in magnetic separator for 1 minute.
10. Remove and save supernatant in another tube for B lymphocyte isolation.
11. Resuspend particles/cells in 1 mL 20% HIFCS/PBS. Gently flick tube to resuspend particles. Place in magnetic separator for 30 seconds. Discard supernatant with a disposable pipette. Repeat 2 times.
12. Proceed to the "Labeling and Cell Concentration Procedures" (below), or resuspend cells (particles) in 0.5 mL McCoy's medium or equivalent with 5% HIFCS.

C. Isolation from Frozen Ficoll Interface

1. Thaw whole cells at 56°C (DMSO removal is not required).
2. Layer 0.5 mL of cell suspension over 0.5 mL of 50% Percoll in a 1.5 mL centrifuge tube.
3. Centrifuge at 2000 g for 2 minutes, or at 400 g for 10 minutes.
4. Discard supernatant with disposable pipette.
5. Resuspend cells in 1 mL of 20% HIFCS/PBS.
6. Dispense 100 µL of magnetic particles into sample tube and cap tube.
7. Rotate sample for 3 minutes at 20-25°C.
8. Uncap and place tube in magnetic separator for 1 minute.
9. Transfer supernatant to another centrifuge tube for B lymphocyte isolation.
10. Resuspend remaining particles/cells in 1 mL of 20% HIFCS/PBS. Gently flick tube to resuspend particles and place on magnetic separator for 30 seconds. Discard supernatant using disposable pipette. Repeat twice.
11. Proceed to the "Labeling and Cell Concentration Procedures" (below), or resuspend cells (particles) in 0.5 mL of McCoy's medium or equivalent with 5% HIFCS.



Protocol 2. LABELING AND CELL CONCENTRATION PROCEDURES

1. Uncap and place tube in magnetic separator for 1 minute. Remove supernatant. Wash cells (particles) twice with PBS.
2. Add 0.5 mL of CFDA (working solution pH 5.5) and mix.
3. Incubate tube horizontally in the dark for 10 minutes at 20-25°C.
4. Magnetically separate (as described above) and wash cells twice with PBS.
5. Resuspend cells in 0.5 mL of McCoy's medium or equivalent with 5% HIFCS.
6. Add 1 µL of cell suspension to a blank well of a Terasaki tray. Check cell count with a fluorescent microscope. Adjust the concentration to 2 x10^6cells/mL (2,000 cells per well).
7. Samples can be transferred to 1.5 mL tubes and stored horizontally at 2-5°C up to 2 days before testing.

## *TECHNOTES*

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Do not use if the particles are clumped. Severe clumping of the beads may indicate deterioration of the product.

10X Developer Buffer should be a slightly colored clear solution. Formation of precipitates may indicate instability or bacterial contamination.