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# PRODUCT INSERT

REF

## FLUOROBEADS®-T AND FLUOROBEADS®-T DEVELOPER

**Catalog #s FB1-25, FB1-40, FB1-100, and FB1-DEV**

**For General Laboratory Use.**

## INTENDED USE

FluoroBeads®-T provide a simple procedure for the isolation of T lymphocytes for use in HLA Class I typing assays using fluorescent dyes. FluoroBeads®-T Developer is specifically designed to be used in the FluoroBeads®-T isolation method.

## SUMMARY AND EXPLANATION

FluoroBeads®-T are immunomagnetic beads which are less than 1 micron in diameter. The anti-CD2 monoclonal antibodies coupled to the bead surface specifically bind to the E-rosette receptor on T lymphocytes. FluoroBeads®-T offer the user a quick method of separating T lymphocyte/FluoroBeads®-T complexes from blood with the use of a collector magnet. The FluoroBeads®-T method requires no cold incubations, rotations, or centrifugations. FluoroBeads®-T Developer is a reagent specifically designed to enhance the performance of FluoroBeads®-T immunomagnetic beads.

## PRINCIPLE

Immunomagnetic beads are superparamagnetic particles with monoclonal antibodies coupled to their surface. The beads can be collected using a magnetic field. When the magnetic field is removed, the beads do not retain any residual magnetism. They can be repeatedly magnetized and redispersed. The specificity of the coupled monoclonal antibody determines the type of cell collected.

## REAGENTS

**A. Identification**

FluoroBeads® are superparamagnetic particles coupled to monoclonal antibodies, and suspended in PBS with stabilizers and sodium azide as a preservative. The monoclonal antibodies are of murine origin. The FluoroBeads Developer contains non-toxic proprietary ingredients.

**B. Warning or Caution**

1. **WARNING:** All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested in accordance with current FDA required tests. No known test method can offer assurance that products derived from human blood will not transmit infectious agents.
2. **WARNING:** This reagent contains 0.01% sodium azide which under acidic conditions yields hydrazonic acid, an extremely toxic compound. Reagents containing sodium azide should be diluted in running water prior to being discarded. These conditions are recommended to avoid deposits in plumbing where explosive conditions may develop.
3. **CAUTION:** Do not use lithium heparin or EDTA as an anticoagulant for your blood sample.
4. Refer to Material Safety Data Sheet for detailed information.

**C. Instructions for Use**

See DIRECTIONS FOR USE on page 3.

**D. Storage Instructions**

Store reagents at temperature indicated on package. Use before printed expiration date.

**E. Purification or Treatment Required for Use**

**FluoroBead®s-T:** Resuspend before use by vortexing for 10 seconds.

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

**F. Instability Indications**

**FluoroBeads®-T:** Do not use if the beads are clumped. Severe clumping of the beads may indicate deterioration of the product.

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

## SPECIMEN COLLECTION AND PREPARATION

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).

## PROCEDURE

**A. Materials Provided**

1. FluoroBeads®-T
2. Instructions for cell isolation and testing
3. FluoroBeads®-T Developer (10X). See "Purification or Treatment Required for Use" for 1X instructions.

**B. Materials Required, But Not Provided**

1. Class I tissue typing trays (One Lambda or equivalent)
2. 5 ml and 1.5 ml plastic or glass centrifuge tubes with caps
3. Phosphate Buffered Saline (PBS) without $Ca^{++}$ and $Mg^{++}$ salts (*i.e.*, Irvine Scientific Cat. #9242)
4. McCoy's medium or equivalent with 5% HIFCS
5. Magnetic separator (One Lambda or equivalent)
6. Aspirator or disposable pipettes
7. 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.

**C. Optional Materials Not Provided**

1. Ficoll-Hypaque
2. 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.
3. Stain/Quench Reagents:
   a) Acridine Orange/Ethidium Bromide FluoroQuench™ (OLI Cat. #FQAE500), or
   b) Ethidium Bromide FluoroQuench™ (OLI Cat. #FQEB500), or
   c) Add 1 ml EB stock solution to 9 ml hemoglobin or 1% ink (See Materials #s 11 - 14).
4. Hemoglobin: Dissolve 10 gm lyophilized hemoglobin in 90 ml 5% EDTA/PBS. Bring volume to 99 ml. Add 1 ml of 1% sodium azide. Centrifuge at 1000 g for 45 minutes. Store supernatant at -20°C.
5. Ink: Dissolve 1 gm bovine serum albumin (BSA) in 10 ml 5% EDTA/PBS add 0.1 ml 1% sodium azide and 0.1 ml Higgins Black Calligraphy Ink.
6. 1% Sodium Azide: Dissolve 1 gm sodium azide in 100 ml PBS.
   ***Caution:*** *Sodium Azide is toxic. Always wear protective equipment when handling.*
7. 5% Disodium Ethylenediamine-tetraacetic Acid (EDTA)/PBS: Add 5 gm EDTA to 90 ml PBS. Adjust pH to 7.2 with 10 M NaOH to dissolve EDTA. Bring volume to 100 ml with PBS.
8. Ethidium Bromide (stock solution): dissolve 50 mg in 1 ml distilled water. Add 49 ml PBS. Heat in water bath at 56°C for 30 min. Aliquot and freeze at -20°C.
9. 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.

**D. Step-by-step procedure.**

See DIRECTIONS FOR USE on page 3.

## DIRECTIONS FOR USE

### ISOLATION TECHNIQUES

**A. Isolation from Whole Blood**

1. Dispense 2 ml of blood into a 5 ml tube.
2. Resuspend FluoroBeads®-T thoroughly before use. **Vortex approximately 10 seconds**
3. Add 100 µl FluoroBeads®-T to blood sample. Immediately cap tube and invert 2 – 3 times to disperse magnetic beads.
4. Rotate tube once per second for 3 minutes at 20 – 25°C to allow binding of beads 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 (see "Treatment Required for Use" instructions above). 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 (beads) with 1 – 2 ml PBS. Gently flick tube to disperse beads. 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 (beads) 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 FluoroBeads®-T 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 with FluoroBeads®-B.
11. Resuspend beads/cells in 1 ml 20% HIFCS/PBS. Gently flick tube to resuspend beads. 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 (beads) 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 FluoroBeads®-T 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 with FluoroBeads®-B.
10. Resuspend remaining beads/cells in 1 ml of 20% HIFCS/PBS. Gently flick tube to resuspend beads 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 (beads) in 0.5 ml of McCoy's medium or equivalent with 5% HIFCS.

### LABELING AND CELL CONCENTRATION PROCEDURES

**A. CFDA Method**

1. Uncap and place tube in magnetic separator for 1 minute. Remove supernatant. Wash cells (beads) 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 x $10^6$cells/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.

**B. FQAE Method**

1. Add 1 µl of cells (beads) to a well of a Terasaki tray.
2. Add 5 µl of FQAE (OLI Cat. #FQAE-500) to well.
3. Check cell count with a fluorescent microscope. Adjust cell concentration to 2 x 106cells/ml (2,000 cells per well).
4. Samples can be transferred to 1.5 ml tubes and stored horizontally at 2 - 5°C up to 2 days before testing.

**ABC TYPING**

**A. CFDA Method**

1. Mix cell preparation by tapping pellet and inverting tube. Do not mix with a syringe. Add 1 µl of CFDA labeled cells (beads) to each well of an HLA Class I typing tray.
2. Incubate in the dark for 30 minutes at 20 - 25°C. **(For OLI LMT™, incubate for 60 minutes at 20 - 25°C and proceed to Step #5)**.
3. Add 5 µl of ABC rabbit complement to each well.
4. Incubate the tray in the dark for 1 hour at 20 - 25°C.
5. To each well, add 5 µl of **one** of the following quenching reagents:
   a) FluoroQuench™ Ethidium Bromide (OLI Cat. #FQEB500), or
   b) EB/hemoglobin working solution, or
   c) EB/1% ink working solution.
6. Trays can be read immediately or stored in the dark at 2 -5°C for up to 2 days.

**B. FQAE Method**

1. Mix cell preparation by tapping pellet and inverting tube. Do not mix with a syringe. Add 1 µl cells (beads) to each well of an HLA Class I typing tray.
2. Incubate for 30 minutes at 20 - 25°C. (For OLI LMT™, incubate for 60 minutes at 20 - 25°C and proceed to Step #5)
3. Add 5 µl ABC complement to each well.
4. Incubate for 1 hour in the dark at 20 - 25°C.
5. To each well, add 5 µl of FQAE.
6. Trays can be read immediately or stored in the dark at 2 - 5°C for up to 2 days.

## LIMITATIONS OF THE PROCEDURE

The cell yield will vary with each specimen depending on the cell count and the time since blood collection. Various diseases can cause a decrease in the lymphocyte yield. Some medications can cause a decrease in the lymphocyte yield as well, and may cause a decrease in HLA antigen expression. Cadaveric samples may have low lymphocyte yields with elevated monocyte and granulocyte contamination.

Contamination with other cells can cause weak/false negative reactions. Monocytes have a variable amount of HLA Class I and Class II antigens. Platelets have HLA Class I antigens and can weaken antisera by absorbing the antibodies from the antisera.

## EXPECTED VALUES

FluoroBeads®-T contain enough immunomagnetic beads to isolate more than 90% of the CD2+ T cells in 1 ml of whole blood.

## SPECIFIC PERFORMANCE CHARACTERISTICS

The purity of the lymphocytes isolated should be over 90%. Cells should be reactive with anti-HLA sera, and be lysed under standard lymphocytotoxicity assay conditions.

## EC REP EUROPEAN AUTHORIZED REPRESENTATIVE

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## REVISION HISTORY

| Revision | Date | Revision Description |
|---|---|---|
| 0 | 2007/04 | Clarify which anticoagulants are not acceptable. Update MDSS address. |