

PRODUCT INSERT



FLUROBEADS[®]-B AND PHOSPHATE BUFFERED SALINE (PBS)/CITRATE REAGENT Catalog # FB-25, FB2-40, FB2-100, PC1-500

For General Laboratory Use.



INTENDED USE

FluroBeads[®]-B provide a simple procedure for the isolation of B lymphocytes for use in HLA Class II typing assays using fluorescent dyes. PBS/Citrate is a reagent for use in the FluroBeads[®]-B isolation method.

SUMMARY AND EXPLANATION

FluroBeads[®]-B are immunomagnetic beads that are less than 1 micron in diameter. Anti-CD19 monoclonal antibodies are coupled to the bead surface. CD19 antibodies specifically bind to B lymphocytes. FluroBeads[®]-B offer the user a quick method of separating B lymphocytes from blood with the use of a collector magnet. The FluroBeads[®]-B method requires no cold incubations, rotations, or centrifugations. PBS/Citrate enhances the performance of the beads.

PRINCIPLE(S)

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

FluroBeads[®]-B are superparamagnetic particles coupled to anti-CD19 monoclonal antibody and suspended in BSA/PBS with NaN₃ as a preservative. The monoclonal antibody is of murine origin. The PBS/Citrate reagent contains citric acid, Na₃ citrate, PBS, and other proprietary ingredients.



B. Warning or Caution

1. For General Laboratory Use.
2. **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.
3. **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.
4. **CAUTION:** Do not use lithium heparin as an anticoagulant for your blood sample.
5. Refer to the 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

Resuspend FluroBeads[®]-B thoroughly before use by vortexing approximately 10 seconds.

F. Instability Indications

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

SPECIMEN COLLECTION AND PREPARATION

Approximately 10 ml of whole blood should be drawn. The preferred anticoagulant is ACD or CPDA. **Do not use lithium heparin!** B 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 to 25° C) until beginning the isolation procedure.

PROCEDURE

A. Materials Provided

1. FluoroBeads® -B
2. Instructions for cell isolation and testing

Materials Required, But Not Provided

1. Class II 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. #9249)
4. McCoy's medium or equivalent **with 5% HIFCS**
5. Magnetic Separator (One Lambda or equivalent)
6. Aspirator or disposable pipettes
7. PBS/Citrate Reagent
 - a. OLI Cat. #PC1-500
 - b. PBS/Citrate Reagent
 - 1) Stock Solution (10X Citrate): Dissolve 7 gm Trisodium Citrate Dihydrate and 2.5 gm Citric Acid in 90 ml distilled water. Bring final volume to 100 ml. Filter, sterilize, and store at 2° to 5° C.
 - 2) Working Solution (1X PBS/Citrate): Add 10 ml of 10X Citrate to 90 ml of PBS. Filter, sterilize, and store at 2° to 5° C.
- 8) Heat-Inactivated Fetal Calf Serum (HIFCS)
 - a. Stock solution: Heat FCS at 56° C for 30 minutes to inactivate complement. Store at 2° to 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.

B. 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 [Density (D) = 1.077].
 - 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 #12-16)

CAUTION: Sodium Azide is toxic. Always wear protective equipment when handling Sodium Azide.

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.
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 to 56° C for 30 minutes. 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 the 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 to 5° C for up to one week.

- Prepared in PBS at pH 5.5: Add 30 μ l stock CFDA solution to 5 ml PBS (pH 5.5). Store at 2 to 5° C for up to one week.

Step-by-step procedure.

See DIRECTIONS FOR USE below.

**DIRECTIONS FOR USE
ISOLATION TECHNIQUES**

A. Isolation from Buffy Coat

1. Centrifuge whole blood at 400 to 900 g for 10 minutes.
2. Transfer approximately 1 ml of buffy coat to a 5 ml tube.
3. Add 4 ml PBS/Citrate.
4. Resuspend FluoroBeads[®]-B thoroughly before use. Vortex approximately 10 seconds.
5. Add 100 μ l FluoroBeads[®]-B to blood sample. Immediately cap tube and invert 2 to 3 times to disperse magnetic beads.
6. Rotate tube once per second for **3 minutes** at 20° to 25° C to allow the beads to bind B cells. Do not exceed 4 minutes. Use an end-over-end rotating device or hand mix.
7. Uncap and place tube in magnetic separator for 2 minutes. Do not exceed 3 minutes.
8. Remove and discard supernatant with a disposable pipette. Remove tube from magnet.
9. Resuspend cells (beads) with 1 to 2 ml PBS/citrate. Gently flick tube to disperse beads. Replace tube in magnetic separator for 1 minute. Remove and discard supernatant. Repeat twice using PBS only.
10. Proceed to the “Labeling and cell Concentrations Procedures” (below), or resuspend cells (beads) in 0.5 ml of McCoy’s medium or equivalent medium with 5% HIFCS.

B. Isolation from Ficoll Interface

1. Centrifuge citrated or heparinized blood for 10 minutes at 400 to 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 **1,000 g**.
4. Collect approximately 1 ml of interface from each tube and transfer into centrifuge tubes. Centrifuge for 1.5 minutes at **3,000 g** or 10 minutes at **1,000 g**.
5. Discard the supernatant and 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 μ l FluoroBeads[®]-**B** to sample tube and cap the tube.
8. Rotate sample once per second for 3 minutes at 20° to 25° C.
9. Uncap and place tube in magnetic separator for 1 minute.
10. Remove and save supernatant in another tube for T lymphocyte isolation with FluoroBeads[®]-T.
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 two times.
12. Proceed to “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 37° C (DMSO removal is not required).
2. Layer 0.5 ml of cell suspension over 0.5 ml 50% Percoll in a 1.5 ml centrifuge tube.
3. Centrifuge at 2000 g for 2 minutes or a 400 g for 10 minutes
4. Discard supernatant with disposable pipette.
5. Resuspend cells in 1 ml 20% HIFCS/PBS.
6. Dispense 100 μ l FluoroBeads[®]-B into sample tube and cap tube.
7. Rotate sample once per second for 3 minutes at 20° to 25° C.
8. Uncap and place tube in magnetic separator for 1 minutes.
9. Transfer supernatant to another centrifuge tube for T lymphocyte isolation with FluoroBeads[®]-T.
10. Resuspend remaining beads/cells in 1 ml 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 McCoy’s medium or equivalent with 5% HIFCS.

D. Isolation from Whole Blood

1. Transfer 5 ml whole blood to a 15 ml tube.
2. Add 5 ml 1X PBS/Citrate and mix by inversion.
3. Dispense 100 µl FluoroBeads[®]-B into the sample and rotate once per second for 5 minutes (do not rotate more than 5 minutes) with either an end-over-end rotator or by hand at 20° to 25° C.
4. Uncap and place tube in magnet for 5 minutes. Remove and discard supernatant. Remove tube from magnet.
5. Add 2 to 3 ml of 1X PBS/Citrate to sample. Gently flick to resuspend beads. Place tube in magnet for 1 minute. Repeat twice using PBS only.
6. Proceed to "Labeling and Cell Concentration Procedures" below, or resuspend cells (beads) in 0.5 ml 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. Resuspend cells (beads) with PBS. Repeat twice.
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° to 25° C.
4. Repeat Step 1 above.
5. Resuspend cells in 0.5 ml McCoy's medium or equivalent with 5% HIFCS.
6. Add 1 µl cell suspension to a blank well of a Terasaki tray. Check cell count with a fluorescent microscope. Adjust the concentration to 2×10^6 /ml (2,000 cells per well).
7. Samples can be transferred to 1.5 ml tubes and stored horizontally at 2° to 5° C up to 2 days before testing.

B. FQAE Method

1. Add 1 µl cells (beads) to a blank well of a Terasaki tray.
2. Add 5 µl FQAE (OLI Cat. #FQAE-500) to well.
3. Check cell count with a fluorescent microscope. Adjust cell concentration to 2×10^6 /ml (2000 cells per well).
4. Samples can be transferred to 1.5 ml tubes and stored horizontally at 2° to 5° C up to 2 days before testing.

DR TYPING PROCEDURES

Note: The following are recommended protocols. Incubation times may vary depending on the strength of typing reagents and/or complement used. (Thirty minutes with antibody and sixty minutes with complement is suggested for One Lambda tissue typing reagents.)

A. CFDA Method

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

6. Trays can be read immediately, or may be stored in the dark at 20° to 25° 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 yields, 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®-B contain enough immunomagnetic beads to isolate more than 90% of the CD19⁺ B cells in a buffy coat from 10 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 should be lysed under standard lymphocytotoxicity assay conditions.

EC REP EUROPEAN AUTHORIZED REPRESENTATIVE

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REVISION HISTORY

Revision	Date	Revision Description
7	2007/05	Delete statement that "Granulocytes are negative for HLA antigens." Editorial review with minor formatting, punctuation and grammar corrections. Update template and MDSS address.
8	2008/05	Corrected typo in centrifuge in G-force settings.

