



CytoFuge®

Application Note #1 Buffy Coat Slide Preparation

Purpose

To prepare a uniform deposit of white blood cells suitable for staining and analyzing.

Material required:

- CytoFuge and 3-chamber cell concentrators
- SafeCrit plastic hematocrit tubes, untreated
- Hematocrit centrifuge, sealant pad
- Tube cutter or razor-blade type knife
- Microscope slides - high quality
- Saline solution or phosphate buffered saline (PBS) 0.01M phosphate buffered 0.14M NaCl, pH 7.3
- 30% bovine serum albumin (BSA) solution - Gamma Biologicals
- 12 x 75 mm plastic polystyrene tubes
- Adjustable pipettor with tips
- Fine-tipped plastic transfer pipets - (StatSpin Re-order No. PF35)
- Not required but most useful - StatSpin microSIP vacuum aspirator

Procedure:

1. Fill, seal and spin (as for a hematocrit) SafeCrit tube. Use untreated tubes for whole blood collected in EDTA treated vacuum tubes.
2. Isolate the buffy coat by cutting the SafeCrit tube just above and below it; no more than 2mm above and below the plasma, red blood cell interface. Use a tube cutter (e.g. pet toe-nail cutter) or notch the tube by rolling on a hard surface against a sharp blade. The tube can then be separated exactly along the notch by bending.
3. Place the buffy coat section into 200 μ L of saline in a 12x75mm tube. Agitate gently to wash out the cells. Repeated gentle aspiration and release using a small pipette will also help resuspend the cells.
4. Further dilute the buffy coat suspension according to the cell content. Dilute with saline or buffered saline (PBS) containing BSA (30 μ L 30% BSA per 600 μ L). The BSA is essential for maximizing cell adhesion to the slide.

Examples: For a WBC count of 1.0×10^3 add six (6) drops (approx. 90 μ L) to 0.6 mL of saline or buffered saline containing BSA.

For a very low WBC count, such as 0.1×10^3 add 180 μ L of suspension to the 0.6 mL of BSA-saline.

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An IRIS Company

85 Morse Street
Norwood, MA
02062 USA

Tel: 781-551-0100
800-782-8774
Fax: 781-551-0036

Procedure (cont.)

5. Assemble a 3-chamber cell concentrator using highest quality precleaned slide. Slides treated with di-amino silane or poly-L-lysine are also acceptable.
6. Add 200 μ L of the diluted cell suspension. Using a smaller volume may result in uneven cell distribution.
7. At the end of the cycle remove the cell concentrators while maintaining the "rest" position. Remove all the residual fluid with a fine-tipped pipet or a pipeting device. The microSIP provides a most convenient way of doing this. Remove the fluid both from the sump and from around the gasket area as well.
8. Disassemble the concentrator and air dry completely.
9. Fix and stain per your standard laboratory procedure.

Helpful hints

The StatSpin microSIP (mini vacuum aspiration system) provides a most convenient method for removing the supernatants from either the 3-chamber or the single-chamber cell concentrator. Disposable fine-tipped plastic transfer pipets (such as StatSpin Re-order No. PF35) provide a means of removing supernatants as well.

Thoroughly read the CytoFuge Operator's Manual.

Some procedures will require the use of surface treated slides such as those treated with di-amino silane (CMS "plus" slides #310-116) or poly-L-lysine treated slides available from many sources. All procedures will benefit from using treated slides.

A method to help locate the cell deposits on the slide: after completing the assembly of either kind of concentrator outline the deposit area with a wax pencil or fine-tipped marker on the back of the slide. The choice of marker will depend on which will wash off during your staining procedure. Use the hole in the backing plate as the guide when lightly outlining the area.

Be sure to remove all the residual fluid, both in the sump and that trapped on the surface of the silicone gasket.



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Application Note #2

Immobilizing cells from CSF (cerebrospinal fluid)

Purpose

To prepare a uniform deposit of cells from CSF fluid (cerebrospinal fluid) suitable for staining and analyzing

Material required:

- CytoFuge and 3-chamber and/or 1-chamber cell concentrators
- Microscope slides - high quality
- 30% bovine serum albumin (BSA) solution - Gamma Biologicals
- adjustable pipettor with tips
- Fine-tipped plastic transfer pipets - (StatSpin Re-order No. PF35)
- Not required but very useful - StatSpin microSIP vacuum aspirator

Procedure:

1. Assemble the cell concentrator following instructions in the Operator's Manual.
2. Outline the deposit area on the underside of the slide with a wax pencil using the opening in the backing plate as a guide.
3. Add 30% BSA solution: 30 μ L from a pipettor or two small drops from a fine-tipped disposable plastic pipet (e.g. StatSpin Re-order No. PF35). Add to each chamber of the 3-chamber concentrator to be used. To the single-chamber concentrator add 90 μ L or six drops with a fine-tipped pipet.
4. Deliver the BSA solution close to the slide surface. The role of the BSA solution is to promote cell recovery and adhesion to the slide.
5. Add the sample to the sump of the cell concentrator while in the "rest" position. For fluids with cell counts of 10-100/ μ L, 200 μ L of sample will yield acceptable cell distribution. For sample with counts higher than 100/ μ L a proportionally smaller sample or a diluted sample is better.

Note: For sample with counts less than 10/ μ L the largest sample may be spun, the supernatant aspirated and the chamber reloaded with additional fluid and respun to increase the number of cells on the deposit.

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Note: While the 3-chamber concentrator and 1-chamber concentrator will hold 450 μ L and 1.8 mL respectively, those liquid volumes will contact the slide before it is brought to the operating position during centrifugation possibly resulting in poor cell distribution on the slide. Consult the Operator's Manual for further information. The nominal maximum volumes added are 200 μ L and 800 μ l respectively.

6. Install the concentrators in the rotor in a balanced manner while maintaining the "rest" position. Attach the rotor cover and spin at 2,200 rpm for 4 minutes.
7. At the end of the cycle remove the cell concentrators while maintaining the "rest" position. Remove all the residual fluid with a fine-tipped pipet or a pipeting device. The microSIP provides a most convenient way of doing this. Remove the fluid both from the sump and from around the gasket area as well.
8. Disassemble the cell concentrator and allow to air dry. Stain with Wright's stain following your laboratory's standard procedure. If that procedure does not involve a fixation step the use of treated slides may be required to assure cell adhesion and retention.



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Application Note #3

Cell Immobilization on poly-L-lysine Treated Slides

Purpose:

The CytoFuge with its 3-chamber cell concentrators is ideal for cell immobilization studies. Three separate samples can be applied on the same slide for further analysis by immunocytochemistry or cell surface marker studies by ELISA methods. As many as four slides can be prepared at once providing 12 wells for testing. Immobilizing the same or different cell populations on the same slide allows exposure to identical staining conditions. In this procedure protein assays were used to measure cell adhesion; washed human platelets were the cell suspension.

Material required:

- CytoFuge and 3-chamber cell concentrators
- Poly-L-lysine treated microscope slides
- 30% bovine serum albumin (BSA) solution - Gamma Biologicals
- Protein assay reagent - bicinchonic acid reagent (BCA) - Pierce
- Phosphate buffered saline (PBS) 0.01M phosphate buffered 0.14M NaCl, pH 7.3
- Adjustable pipettor with tips
- Fine-tipped plastic transfer pipets (StatSpin Re-order No. PF35)
- Not required but very useful - StatSpin microSIP vacuum aspirator
- Cell suspension (e.g. washed platelets; cell count, approx. 200K/ μ L)
- Microplates and plate reader

Procedure:

1. Assemble the 3-chamber concentrators with the treated microscope slides.
2. Add 200 μ L of suspension to the sump of each cell concentrator chamber while in the "rest" position. Load in the rotor, attach rotor cover and spin for 4 minutes at 2,200 rpm.
3. Aspirate the residual fluid.
4. Wash the cell deposits with PBS while the cell concentrators lie flat in a horizontal orientation. Use 200 μ L aliquots and aspirate residual fluid after each addition. (No difference was seen between three and twelve washes.)
5. Prepare the BCA reagent by adding one part Reagent B to fifty parts Reagent A. Add 200 μ L aliquots to each washed chamber and incubate at room temperature for 15-30 minutes. The cell concentrators should be lying flat.

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Procedure (cont.)

6. Remove 50 μL aliquots of the reagent to wells of a microplate and read at 550 nM with an ELISA plate-reader.
7. Prepare a standard curve to quantify cell deposition. Duplicate 5 μL aliquots of PBS (negative control) or 5 μL of platelet suspension or serial 1:2 dilutions of the platelet suspension are added to 45 μL of complete BCA reagent in microplate wells. Incubate simultaneously with the platelet coated slides; read absorbance at 550 nM.

Results:

Increased number of washes (3, 6 or 12, step 4 above) had no effect showing that the cells were firmly attached to the slides. Good reproducibility was achieved. The RSDs between chambers on the same slide was an average 3.4% with a range of 1.6 to 4.7 with $n=24$. Relative Standard Deviations between chambers on different slides was an average 8.2%, range 5.4 to 10.7, $n=8$.



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Application Note #4 Use of Concentrators as Staining Chambers using an ELISA Method

Purpose:

Demonstration of utility of 3-chamber cell concentrators as staining chambers with a typical two-antibody ELISA assay.

Material required:

- CytoFuge and 3-chamber cell concentrators
- Poly-L-lysine treated microscope slides
- Glutaraldehyde solution, 0.5%
- Sodium hydroxide solution, 0.1N
- ELISA buffer: 10 mM TRIS, pH 8.5; 0.14 M NaCl; 1 mM MgCl₂; 1 mM ZnCl₂; 0.05% Tween20
- Antithrombocyte antibody - rabbit anti-human Thrombocyte IgG 20 mg/ml in 0.1 M NaCl, 0.015M Na₃, 1/1000 - Accurate Chem. & Scientific
- Alkaline phosphatase labeled secondary antibody - goat anti-rabbit Fab'2 - 1/500 - Organon Teknika
- Alkaline phosphatase substrate - p-nitrophenyphosphate (0.05M)
- Neutralizing reagent - 10 mM glycine in 0.1% BSA
- Adjustable pipettor with tips
- Fine-tipped plastic transfer pipets (StatSpin Re-order No. PF35)
- Not required but very useful - StatSpin microSIP vacuum aspirator
- Cell suspension (e.g. washed platelets; approx, 200 K/ μ L)
- Microplates and plate reader

Procedure:

1. Immobilize 200 μ L of washed platelets on poly-L-lysine treated slides (or fresh CMS SuperFrost Plus®) by centrifuging for four minutes at 2,200 rpm (See CytoFuge Application Note number 3 for more detail)
2. Aspirate residual fluid and wash the deposits 3 x with ELISA buffer; then fix with 0.5% glutaraldehyde for 15 minutes. Neutralize with 10mM glycine in 0.1% BSA for 30 minutes at 37°C. Wash 2 x with PBS.

Procedure (cont.)

3. Add 150 μ L of primary antibody or control antibody and incubate 60 minutes at 37°C. The cell concentrators should be lying flat for these washes and incubations. (NB. as little of 100 μ L of fluid will adequately cover the surface.)
4. Aspirate the residual fluid and wash the deposits 3 x with buffer.
5. Add 150 μ L of enzyme-labeled secondary antibody and incubate for 60 minutes at 37°C.
6. Aspirate the residual fluid and wash the deposits 3 x with buffer; then 2 x with distilled water.
7. Add 150 μ L of substrate and incubate for 60 minutes at 37°C. Stop the reaction by adding 150 μ L of 0.1N NaOH.
8. Transfer 50 μ L aliquots in duplicate to 96-well microplates and read at 405 nM with a plate reader.

Results:

Excellent agreement between chamber to chamber and slide to slide was found. RSDs for the same slide were average 3.3%, range of 1.2 to 5.8; n=8. Relative Standard Deviations for the slide to slide data averaged 6.1%, range of 4.3 to 10.4; n=4.