NH_4CL LEUKOCYTE SEPARATION KIT
For Laboratory Use

CATALOG NO: 3243

STORAGE TEMPERATURE: 2° to 8°C

MATERIALS PROVIDED:

1. Erythrocyte Lysis Buffer - (Catalog No. 5122) One bottle containing 220 mL of buffered ammonium chloride. (NH_4CL)
2. Fixation Solution (5X) - (Catalog No. 5113) One bottle containing 220 mL of PBS, formalin, sucrose and 0.5% sodium azide.
3. Permeabilization Solution (5X) - (Catalog No. 5115) One bottle containing 220 mL of PBS, Nonidet P-40, sucrose, protein stabilizer and 0.5% sodium azide.
4. Phosphate-Buffered Saline (PBS) - (Catalog No. 5087) Three packets of phosphate buffered saline salts.
5. Wash Supplement (100X) - (Catalog No. 5117) One bottle containing 30 mL of protein solution and 0.1% sodium azide.

SUGGESTED PROTOCOLS:

Reagent Preparation:

Prior to specimen processing, prepare the required volume of PBS, Fixation Solution, Permeabilization Solution and Wash Solution as follows:

- PBS - Dissolve the contents of the PBS packet in 950 mL of filtered distilled or deionized water, mix thoroughly and adjust volume to 1 L with filtered distilled or deionized water.

- Fixation Solution (5X) - Shake well before each use ensuring that the stock solution is completely mixed. Dilute 1/5 in filtered distilled or deionized water and mix thoroughly.

- Permeabilization Solution (5X) - Shake well before each use ensuring that the stock solution is completely mixed. Dilute 1/5 in filtered distilled or deionized water and mix thoroughly.

  Note: *The Permeabilization Solution may separate during storage and appear turbid when mixed.*

- Wash Solution - Ensure that the Wash Supplement stock solution is completely mixed. Dilute 1/100 in PBS and mix thoroughly.

All other reagents are provided ready to use.

USA & Canada • +1(800) 437-7500 • Fax: +1 (951) 676-9209
www.millipore.com

upstate | CHEMICON | Linco
THE EXPERTISE OF UPSTATE®, CHEMICON® AND LINCO® IS NOW A PART OF MILLIPORE

Specimen: Vacutainer tube of blood (Heparin, ACD, EDTA).

Ammonium Chloride Leukocyte Separation / Erythrocyte Lysing

1. Allow all reagents to equilibrate to room temperature.
2. Thoroughly mix blood by gentle inversion. Aseptically pipette 2.0 mL of blood into a sterile screw-capped conical centrifuge tube and add 8 mL of Lysis Buffer.
3. Rock blood solution at room temperature for 5 minutes.
4. Centrifuge at 160 x g for 10 minutes at 4°C.
5. Aspirate and discard the supernatant. Take care not to disrupt the cell pellet.
6. Resuspend the cell pellet in 10 mL of the Lysis Buffer.
7. Centrifuge at 160 x g for 10 minutes at 4°C. Aspirate and discard supernatant.

__Note:__ Erythrocyte lysing may not be necessary in samples with few contaminating red blood cells. Erythrocyte lysing procedure should be repeated on samples with heavy contamination of red blood cells.

Slide Preparation:

1. Adjust the cell concentration to 1.0×10^6 cells/mL in PBS.
2. Using the cytocentrifuge, centrifuge 200 μL (2×10^5 cells) of cell suspension onto a glass cytocentrifuge slide (prepare at least 3 slides per specimen) at 600-900 RPM for 3 to 4 minutes.
3. Remove slides from the centrifuge, allow the slides to air dry, and fix immediately.

Fixation:

1. Fix slides in Fixative Solution for 10 minutes at room temperature.
2. Transfer slides to a Coplin jar containing Wash Solution.
3. Repeat wash procedure using fresh Wash Solution.

Permeabilization:

1. Permeabilize cells by immersing the fixed slides in Permeabilization Solution for 5 minutes at room temperature.
2. Place slides in fresh Wash Solution then rinse in distilled or deionized water.
3. Rapidly air dry slides.
4. Stain immediately or store at 2° to 8° C for up to 18 hours. Slides may be stored desiccated at <-70°C for up to 12 months.

Control Slide Production:

Control slides should be prepared from known positive and negative blood samples following the Slide Preparation, Fixation, and Permeabilization procedures. Control slides may be stored desiccated at
< -70° C for up to 12 months.

References:

1. Landry, M.L. and Ferguson, D. (1993). Comparison of quantitative cytomegalovirus antigenemia assay with culture methods and correlation with clinical diseases. J. Clin Micro 31: 2851-2856.
2. Ho et al; (1998) Rapid cytomegalovirus pp65 antigenemia assay by direct erythrocyte lysis and immunofluorescence staining. J. Clin Micro 36: 638-640.

©2002 - 2009: Millipore Corporation. All rights reserved. No part of these works may be reproduced in any form without permission in writing.

USA & Canada • +1(800) 437-7500 • Fax: +1 (951) 676-9209
www.millipore.com