



Tech Tips

Xenographic Retention Chromatography (XRC)

Preparing & Preserving Mouse Whole Blood Specimens

Overview

This is a convenient procedure for preparing and preserving mouse whole blood specimens for assaying with the EpiSep™ Cytokeratin Kits.

Reagents Required

Refer to the sample worksheet on Page 2 for total reagent volume requirements.

- Fixative - 0.05% Glutaraldehyde-PBS solution (preparation procedure below).
- 25% Glutaraldehyde.
- **Cold** 0.01M PBS, pH 7.4, use PBS stored at 4°C, if possible.
- 0.1% BSA Solution (preparation procedure below).
- Blocking Buffer (Cat.# R2116-1).
- 0.01M PBS Solution

Reagent Preparation

Refer to the sample worksheet on Page 2 for dilution volume calculations.

- 1) Prepare 0.05% Glutaraldehyde-PBS Fixative solution fresh on the day of use.
 - a) Use **cold** 0.01M PBS, pH 7.4, stored at 4°C.
 - b) Dilute 25% glutaraldehyde 1:500 in **cold** 0.01M PBS, pH 7.4.
- 2) Prepare 0.1% BSA Solution
 - a) Dilute Blocking Buffer 1:10 using 0.01M PBS solution.

Suggested Sample Preparation Procedure

- 1) Centrifuge 50µL – 1mL of whole blood for 5 minutes at 500xg.
 - a) For small sample volumes, less than 100µL, dilution of the specimen with **cold** 0.01M PBS, pH 7.4, should be considered before centrifugation.
- 2) Remove supernatant using a transfer pipette.
- 3) Add 4mL of 0.01M PBS, pH 7.4 to the pellet.
- 4) Resuspend pellet by gentle inversion or by placing on Microvial Rotator for 5 – 10 minutes.
- 5) Centrifuge solution for 5 minutes at 500xg.
- 6) Remove supernatant as completely as possible using a transfer pipette.
- 7) Add 4mL of **cold** 0.05% Glutaraldehyde-PBS fixative solution.
- 8) Resuspend cell pellet by placing on Microvial Rotator for 5 – 10 minutes.

- 9) Incubate for 5 minutes while rotating on the Microvial Rotator.
- 10) Centrifuge for 5 minutes at 500xg.
- 11) Remove supernatant using a transfer pipette.
- 12) Wash with 2mL 0.1% BSA Solution.
- 13) Centrifuge for 5 minutes at 500xg.
- 14) Remove supernatant using a transfer pipette.
- 15) Again, wash with 2mL 0.1% BSA Solution.
- 16) Centrifuge for 5 minutes at 500xg.
- 17) Remove supernatant using a transfer pipette.
- 18) Add 500 – 1000µL EpiSep™ Blocking Buffer to the pellet.
- 19) Resuspend by gentle inversion.

For testing within 24 hours store at 2 – 8°C.

Technical Assistance

For technical assistance, contact the WaveSense, Inc. Technical Service Department at (800) 807-7760 or (949) 341-1980 or Fax to (949) 341-1982.

Customer Service

To place an order, contact the WaveSense, Inc. Customer Service Department at (800) 807-7760 or (949) 341-1980 or Fax to (949) 341-1982.

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Worksheets

Important

The calculation sheet below is provided as an example for your convenience. Reagents may be listed more than once to be used either in a dilution step or as a working solution separately.

Reagent Preparation & Requirements Worksheet

Reagents Required	Volume Required per Sample	# of Samples	Total Required Volume of Reagents	Lot #	Expiration Date
Fixative - 0.05% Glutaraldehyde-PBS Solution (1:500) dilution	4.0mL	_____	_____µL		
25% Glutaraldehyde	8µL	_____	_____µL		
Cold 0.01M PBS, pH 7.4	3.992mL	_____	_____µL		
Cold 0.01M PBS, pH 7.4	4mL	_____	_____mL		
0.1% BSA Solution (1:10)	4.0mL	_____	_____µL		
Blocking Buffer	400µL	_____	_____µL		
0.01M PBS Solution	3.6mL	_____	_____µL		
Blocking Buffer	1mL	_____	_____mL		

Suggested Sample Preparation Procedure

Step	Start Time	End Time	Complete ✓
1) Centrifuge 50µL – 1mL of whole blood for 5 minutes at 500xg.			
2) Remove supernatant using a transfer pipette.			
3) Add 4mL of 0.01M PBS, pH 7.4 to the pellet.			
4) Resuspend pellet by gentle inversion.			
5) Centrifuge solution for 5 minutes at 500xg.			
6) Remove supernatant as completely as possible using a transfer pipette.			
7) Add 4mL of cold 0.05% Glutaraldehyde-PBS fixative solution.			
8) Resuspend cell pellet by flash vortexing immediately.			
9) Incubate for 5 minutes while rotating on the Microvial Rotator.			
10) Centrifuge for 5 minutes at 500xg.			
11) Remove supernatant using a transfer pipette.			
12) Wash with 2mL 0.1% BSA Solution.			
13) Centrifuge for 5 minutes at 500xg.			
14) Remove supernatant using a transfer pipette.			
15) Again, wash with 2mL 0.1% BSA Solution.			
16) Centrifuge for 5 minutes at 500xg.			
17) Remove supernatant using a transfer pipette.			
18) Add 500 – 1000µL Blocking Buffer to the pellet.			
19) Resuspend by gentle inversion.			
For testing within 24 hours store at 2 – 8°C.			