



# Tech Tips

## *Xenographic Retention Chromatography (XRC)*

### Preparing & Washing Human Whole Blood Specimens

#### Overview

This is a convenient procedure for preparing and washing human whole blood specimens for assaying with our EpiSep™ Cytokeratin reagent kits.

#### Reagents Required

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

- **Cold** 0.01M PBS, pH 7.4, use PBS stored at 4°C.
- 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 0.1% BSA Solution
  - a) Dilute Blocking Buffer 1:10 using 0.01M PBS.

#### Suggested Sample Preparation Procedure

- 1) Centrifuge 500µL – 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 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) Again, add 4mL of 0.01M PBS, pH 7.4 to the pellet.
- 8) Resuspend pellet by gentle inversion or by placing on Microvial Rotator for 5 – 10 minutes.
- 9) Centrifuge solution for 5 minutes at 500xg.
- 10) Remove supernatant as completely as possible using a transfer pipette.
- 11) Wash with 2mL 0.1% BSA Solution.
- 12) Centrifuge for 5 minutes at 500xg.
- 13) Remove supernatant using a transfer pipette.
- 14) Again, wash with 2mL 0.1% BSA Solution.
- 15) Centrifuge for 5 minutes at 500xg.
- 16) Remove supernatant using a transfer pipette.
- 17) Add 500 – 1000µL Blocking Buffer to the pellet.
- 18) Resuspend by gentle inversion.

**Note: 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
Cold 0.01M PBS, pH 7.4	4.0mL		_____ mL		
0.1% BSA Solution (1:10)	4.0mL	_____	_____ $\mu$ L		
Blocking Buffer	400 $\mu$ L	_____	_____ $\mu$ L		
0.01M PBS Solution	3.6mL	_____	_____ $\mu$ L		
Blocking Buffer	1.0mL	_____	_____ mL		

### Suggested Sample Preparation Procedure

Step	Start Time	End Time	Complete <input checked="" type="checkbox"/>
1) Centrifuge 50 $\mu$ L – 1 mL 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 0.01M PBS, pH 7.4 to the pellet.			
8) Resuspend pellet by gentle inversion.			
9) Centrifuge solution for 5 minutes at 500xg.			
10) Remove supernatant as completely as possible using a transfer pipette.			
11) Wash with 2mL 0.1% BSA Solution.			
12) Centrifuge for 5 minutes at 500xg.			
13) Remove supernatant using a transfer pipette.			
14) Again, wash with 2mL 0.1% BSA Solution.			
15) Centrifuge for 5 minutes at 500xg.			
16) Remove supernatant using a transfer pipette.			
17) Add 500 – 1000 $\mu$ L Blocking Buffer to the pellet.			
18) Resuspend by gentle inversion.			

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