Tech Tips

Xenographic Retention Chromatography (XRC)

Preparing & Preserving Mononucleated Cells

Overview
This is a convenient procedure for preparing and preserving mononucleated cells after they have been isolated using the Ficoll process. This procedure is compatible with our EpiSep™ Cytokeratin reagents kits.

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

- 4% Formaldehyde Solution in 0.01M PBS, pH 7.4.
- 0.01M PBS, pH 7.4.
- Blocking Buffer (Cat.# R2116-1).
- Cell Storage Solution (Cat.# R2129-1).

Suggested Sample Preparation Procedure

1) Centrifuge cell suspension for 5 minutes at 500xg.
2) Remove supernatant using a transfer pipette.
3) Add 1mL of 4% Formaldehyde solution in 0.01M PBS, pH 7.4.
4) Resuspend cells with brief vortexing.
5) Mix the solution by rotating the vial for 5 minutes on the Microvial Rotator.
6) Centrifuge the resuspended sample for 5 minutes at 500xg.
7) Remove supernatant as completely as possible using a transfer pipette without disturbing the pellet.
8) Add 1mL of 0.01M PBS, pH 7.4 to the pellet.
9) Resuspend cell with brief vortexing.
10) Mix the solution by rotating the vial for 3 – 5 minutes on the Microvial Rotator.
11) Centrifuge cell suspension for 5 minutes at 500xg.
12) Remove supernatant using a transfer pipette.
13) Resuspend pellet with 1mL of Blocking Buffer.
14) Incubate the sample for 3 – 5 minutes while rotating on the Microvial Rotator.

Assay the samples within 24 – 48 hours. Otherwise, proceed with the next steps.

15) Centrifuge cell suspension for 5 minutes at 500xg.
16) Remove supernatant using a transfer pipette.
17) Add 1mL of Cell Storage Solution to the pellet.
18) Resuspend pellet by flash vortexing.
19) Store frozen between –10 and –20°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
4% Formaldehyde Solution in 0.01M PBS, pH 7.4*	1mL	_____	_____mL		
0.01M PBS, pH 7.4	1mL	_____	_____mL		
Blocking Buffer	1mL	_____	_____mL		
Cell Storage Solution	1mL	_____	_____mL		

Suggested Sample Preparation Procedure

Step	Start Time	End Time	Complete ✓
1) Centrifuge cell suspension for 5 minutes at 500xg.			
2) Remove supernatant using a transfer pipette.			
3) Add 1mL of 4% Formaldehyde solution in 0.01M PBS, pH 7.4.			
4) Resuspend cells with brief vortexing.			
5) Mix the solution by rotating the vial for 5 minutes on the Microvial Rotator.			
6) Centrifuge the resuspended sample for 5 minutes at 500xg.			
7) Remove supernatant as completely as possible using a transfer pipette without disturbing the pellet.			
8) Add 1mL of 0.01M PBS, pH 7.4 to the pellet.			
9) Resuspend cell with brief vortexing.			
10) Mix the solution by rotating the vial for 3 – 5 minutes on the Microvial Rotator.			
11) Centrifuge cell suspension for 5 minutes at 500xg.			
12) Remove supernatant using a transfer pipette.			
13) Resuspend pellet with 1mL of Blocking Buffer.			
14) Incubate the sample for 3 – 5 minutes while rotating on the Microvial Rotator.			
Assay the samples within 24 – 48 hours. Otherwise, proceed with the next steps.			
15) Centrifuge cell suspension for 5 minutes at 500xg.			
16) Remove supernatant using a transfer pipette.			
17) Add 1mL of Cell Storage Solution to the pellet.			
18) Resuspend pellet by flash vortexing.			
19) Store frozen between –10 and –20°C.			

*Use cold 0.01M PBS, pH 7.4.

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