Protocol for Stabilisation of Lymph Node/Fine Needle Aspirate (FNA) Specimens With TransFix®

1. Place specimen in 1mL phosphate-buffered saline (PBS).
   N.B Specimens must be fresh samples (not resuspended in ethanol or paraformaldehyde) processed within 3 hours of extraction.

2. Disaggregate the sample received using a 1mL syringe (**do not use mechanical disaggregation or enzymatic digestion).** This will improve the yield of cells, preserving the integrity of cell surface antigens.

   2a. For lymph node biopsies: puncture the solid sample with the syringe and introduce the PBS gently inside it, then aspirate the cell suspension. Repeat this process several times until you get a homogeneous suspension of cells.

   2b. For FNA specimens (usually contain very small tissue fragments): homogenise the sample by aspirating and dispensing the specimen several times using the syringe.

3. Centrifuge homogenised sample at 1,100 *g* for 5 minutes at room temperature (18-25°C). Discard the supernatant and resuspend the pellet in 1ml PBS + 0.02% bovine serum albumin (BSA).
   N.B For samples with a presumed low cell count, 500 µl of PBS + 0.02% BSA is sufficient.

4. Perform a cell count on the resuspended sample (100µl) by flow cytometry, using a DNA binding dye and counting beads.

5. Stabilise the specimen by adding 100µl TransFix to 1ml of resuspended sample.
   N.B Use this stabilization method for specimens containing up to 1 x 10^7 total cells. For samples containing ≤1 x 10^5 total cells (resuspended in 500µl PBS + 0.02% BSA), add 25µl TransFix.

6. Mix gently and store at 2-8°C for a minimum of 16 hours.

7. Mix the sample vial thoroughly and place it at room temperature (18-25°C) for at least 15 minutes before staining.

8. Aliquot an appropriate volume of fixed sample and place into flow cytometer tubes to perform a standard stain-lyse-wash (1 wash) protocol for cell surface immunophenotyping.

9. Acquire the sample on the flow cytometer.

*This protocol has been provided from a customer in a clinical hospital who routinely uses TransFix for lymph node/FNA samples. Cytomark does not take liability if TransFix® is used in applications other than those validated by Cytomark. For further information, please contact cytomark@caltagmedsystems.co.uk.

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