

Tissue preparation for surface antigen staining using the Medimachine system

This preparation protocol is useful for fresh or frozen material.

Warning: Handle all biological specimens and materials with which they come into contact as if capable of transmitting infection. Dispose of waste using proper precautions in accordance with state, and local regulations. Never pipette by mouth. Avoid specimen contact with skin and mucous membranes.

1. Dissect a small piece of tissue (10–20 mm³) into 1–2 mm³ portions and moisten with buffer or medium.
2. Prerinse the Medicons twice with 1 ml of buffer or medium
3. Place tissue and 1 mL of buffer or medium in the Medicons. Replace the lid and insert the Medicons into the Medimachine unit.
4. Disaggregate tissue. The duration of disaggregation depends on the tissue type (see Disaggregation Timing Table).
5. Remove the suspension with a syringe through the syringe port on the Medicons.
6. Rinse the Medicons three times with 1 ml of buffer or medium.
7. Filter the suspension through a Filcon with the appropriate pore size (50- μ m Filcons are suitable for most tissues, for example, lymph nodes, tumors, and skin).
8. Rinse the Filcon three times with 1 ml of buffer or medium.
9. Centrifuge the suspension for 5 minutes at 250g.
10. Resuspend the pellet with the appropriate buffer or medium.
11. Repeat steps 3 through 10 if required.
12. Adjust the cell concentration to 5×10^5 to 1×10^6 cells/ml.
13. Incubate 100 mL of suspension for 15–30 minutes at 2°C–8°C with the appropriate concentration of antibody.
14. Add 2 mL of cold buffer or medium (2°C–8°C) and centrifuge for 5 minutes at 250g.
15. Resuspend the pellet in a buffer or medium.
16. Analyze the sample.

Note: The procedure was validated by CTSV but should be adapted to your laboratory and validated for each tissue type you process.



Disaggregation Timing Table

Tissue	Quantity (1–2-mm ³ pieces)	Pulses	Duration	Conditions
Breast	3–4	two (2) times	30 seconds	fresh or frozen
Bladder	4–5	two (2) times	20 seconds	fresh or frozen
Colon	3–6	two (2) times	20 seconds	fresh or frozen
Stomach	4–6	two (2) times	20 seconds	fresh or frozen
Marginal tissue of stomach tumor	3–6	two (2) times	20 seconds	fresh or frozen
Healthy tissue, stomach tumor	3–4	two (2) times	20 seconds	fresh or frozen
Lymph node	3–6	two (2) times	15 seconds	fresh or frozen
Spleen	3–6	two (2) times	15 seconds	fresh or frozen
Liver	3–6	two (2) times	15 seconds	fresh or frozen
Ovary	3–6	two (2) times	30 seconds	fresh or frozen
Prostate	2–3	two (2) times	35 seconds	fresh or frozen
Brain	3–6	one (1) time	10 seconds	fresh or frozen
Lung	2–3	two (2) times	30 seconds	fresh or frozen
Uterus	3–6	one (1) time	60 seconds	fresh or frozen
Skin	3–4	one (1) time	45 seconds	fresh or frozen

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