

## Isolation of DNA

This preparation  
protocol is useful for  
fresh or frozen material.

*Warning: All biological specimens and materials with which they come into contact should be handled as if capable of transmitting infection and disposed of with proper precautions in accordance with federal, 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 mm<sup>3</sup>) into 3–6 mm<sup>3</sup> pieces and moisten with TKM1-NP buffer (10 mM tris-HCl, pH 7.6, 10 mM KCl, 10 mM MgCl<sub>2</sub>, 2 mM EDTA and 2.5 µL/mL of Nonidet P-40).
2. Pre-rinse the Medicon twice with 1 mL of TKM1-NP buffer.
3. Place tissue and 1 mL of TKM1-NP buffer in the Medicon. Replace the lid and insert the Medicon into the Medimachine.
4. Disaggregate tissue. Duration of disaggregation depends on the tissue type (see Disaggregation Timing Table) and will take 2–4 minutes.
5. Remove the suspension with a syringe through the syringe port on the Medicon.
6. Transfer the suspension to a 15 mL Falcon® tube containing 4 mL of TKM1-NP buffer to complete cell membrane lysis.
7. Centrifuge the suspension for 10 minutes at 250 x g at room temperature.
8. Carefully remove the supernatant making sure not to disrupt the pellet.
9. Re-suspend the pellet in 0.8 mL of TKM2 buffer (10 mM tris-HCl, pH 7.6; 10 mM KCl; 10 mM MgCl<sub>2</sub>; 0.4 M NaCl; 2 mM EDTA) and 50 µL of 10% SDS.
10. Thoroughly mix the suspension by pipetting it back and forth.
11. Incubate the sample for 15 minutes at 55°C.
12. Add 0.3 mL of 6 M NaCl to the suspension and mix.
13. Centrifuge the suspension for 10 minutes in a microcentrifuge.
14. Carefully remove and keep the supernatant containing the DNA and discard the pellet.
15. Add two volumes of 100% ETOH and mix the suspension until the DNA has precipitated completely.
16. Further purification can be done according to standard procedures.

NOTE: The procedure was validated by Consul T.S. but should be adapted to your laboratory and validated for each tissue type you process.

## Disaggregation Timing Table

Tissue	Quantity (3-6 mm <sup>3</sup> pieces)	Pulses	Duration	Conditions
Breast	3-6	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, of 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

NOTE: These times were validated by Consults but should be adapted to your laboratory and validated for each tissue type you process.