



Microdissection of heart and other difficult to dissect tissues with Unipick™: an optimized protocol

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This protocol is suitable for the dissection of muscles and other difficult to dissect tissues and isolation of high quality RNA.

Materials:

1. Liberase DL Research Grade (Roche, REF 05-401-160-001), 5mg vial
2. Proteinase inhibitor (Sigma tablets; SigmaFast S8820)
3. Kolliphor® P188 (Sigma 15759) also known as Pluronic F-68
4. 1M sterile CaCl₂
5. Qiagen RNA lysis buffer (RLT) with fresh β-mercaptoethanol
6. **Unipick™**

Reagents:

Prior to day of Microdissection

1. P188 stock solution (15mM; 100x stock solution), place in a 37°C water bath to dissolve, filter sterilize and store at 4°C in the dark

2. Liberase DL Stock Solution:

- a. Slowly thaw the enzyme (lyophilized form) on ice for 30 min
- b. Add 2ml of MEM, alternatively 1x PBS may also be used
- c. Keep on ice, resuspend carefully by pipetting. Do not vortex. Stock may be kept at 4°C for up to a month. Alternatively, aliquot into the smaller volumes (200 µl) and store at -20°C. Use or discard an aliquot after thawing. Do not re-freeze.

Day of Microdissection

1. **Dissociation Solution** 10ml

- a. 200µl Liberase DL stock solution
- b. 75µl 1M CaCl₂
- c. 100µl P188
- d. MEM may be replaced by 1xPBS to 10ml
- e. Place on ice

2. **Inhibitor Solution**

- a. 1 proteinase inhibitor tablet in 250ml MEM or PBS

Tissue Preparation:

1. Perfuse animal with standard PBS (phosphate buffer solution).
2. Remove the heart and other organs and sink in 15-20% Sucrose in PBS at 4°C overnight.
3. Flash freeze heart and organs in 2-methylbutane on dry ice. If tissue is not needed for immediate use, it may be stored at -80°C.
4. Prepare cryosections to desired thickness. (*For hard to dissect tissues, we recommend using 10 to 20 µm thick sections*). Sectioned slides may also be stored at -80°C.
5. Stain slides with 0.025% cresyl violet for 10 seconds, gently wash with PBS.
6. Apply **Dissociation Solution** to tissue sections and incubate at room temperature for no more than 30min.
7. Gently wash tissue sections with **Inhibitor Solution**.

Tissue Microdissection with Unipick™:

1. Dry the back of the slide and place the slide on a microscope stage.

Cover slide surface with **Inhibitor Solution** using a pipettor.

2. Select a disposable capillary unit (DCU) with desired internal diameter (ID) and calibrate it as described in the Calibration section of **Unipick™** manual.
3. Set both dials for vacuum strength and duration to 1, settings can be increased for thicker tissue sections.
4. When the DCU shaft is filled, or when the desired amount of sample has been collected remove the collected sample into a test tube as described in **Unipick™** manual.
5. If some of the collected tissue remains inside the DCU shaft, load the attached syringe with **Inhibitor Solution** to rinse the DCU and to release the remaining contents into the test tube.
6. Spin cells/tissue at 500xg for 5min and remove supernatant (**Inhibitor Solution**).
7. Add appropriate amount of Qiagen RNA lysis buffer (RLT) with fresh β-mercaptoethanol and continue with RNA isolation as described in Qiagen protocol. Samples may then be frozen on dry ice and then stored at -80°C for later use if appropriate.