# RNA Isolation from Laser Capture Microdissection Biological Samples

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## Summary

This protocol is for isolating RNA from laser capture microdissection (LCM) samples captured from PEN-membrane slides. This protocol was originally written on January 2007 and was obtained from Julie Pelletier (UC Davis).

## Materials & Reagents

| Materials/Reagents/Equipment | Vendor | Stock Number |
|---|---|---|
| Full set of micropipettes, 2-1000 µl | Rainin | |
| Thermal Cycler | Many possible | |
| Micro centrifuge | Eppendorf | 5415D |
| Qubit Fluorometer | Invitrogen | |
| Block-heater or water bath | Many possible | |
| Vortex | Many possible | |
| **Consumables** | | |
| Sterilized micropipettes 2-1000 µl | Many possible | |
| Tubes | Ambion | |
| Quant-it RNA assay Kit | Invitrogen | Q32851 |
| Quant-it assay tube | Invitrogen | Q32856 |
| Ethanol | Many possible | |
| RNaqueous-Micro Kit | Ambion | |
| DNase I | Qiagen | |
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## EH&S

JGI employee performing this procedure must wear a lab coat, gloves and safety glasses must be worn when performing this procedure.

## Protocol

**NOTE:** All **reagents/stock solutions** should be **prepared prior** to the start of the procedure.

**1.** Thaw LCM samples and place on ice. Check to see if any crystallization has occurred. If so, add 20ul of lysis buffer to the sample and note the additional volume added.

**2.** Combine the samples into a 1.5ml tube. Rinse the empty 0.2ml tubes with 20-30ul lysis buffer and add to combined samples. Measure the total volume of lysis buffer.

**3.** Calculate what the final volume should be (buffer added to caps while cutting, buffer added later for dissolving the crystals and rinsing the tubes) and the volume of liquid missing. Add RNase free water to 85% of that volume and 15% of lysis buffer. (For example, if the measured volume is 500ul and should be 600ul: add 85ul RNase-free water and 15ul lysis buffer.) Vortex well and spin down briefly.

| | **Sample Name** | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | LCM Collection volume | | | | | | |
| 2 | Rinse Lysis Solution | | | | | | |
| 3 | **Final Volume (1+2)** | | | | | | |
| 4 | Measured Volume | | | | | | |
| 5 | Volume to complete (3-4) | | | | | | |
| 6 | Added Water | | | | | | |
| 7 | Added Lysis Solution | | | | | | |
| | Final Volume (4+6+7=3) | | | | | | |

**4.** Incubate samples in Lysis Solution in water bath at 37°C for 1-2min, then in air incubator at 42°C for 1hour (a minimum of 30minutes). Vortex and spin down briefly.

**5.** Assemble spin column and collection tubes for each samples and label caps. Prewet the filter with 30ul of Lysis Solution and incubate at room temperature for at least 5 minutes, while the two following steps are performed. Centrifuge 30sec at max speed to remove solution.

**6.** Add 3% volume of LCM additive (3ul for 100ul lysate). Vortex and spin down briefly.

**7.** Add 1.25V of 100% ethanol. Mix by pipetting up and own ten times.

| | **Sample Name** | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | LCM Additive (3%) | | | | | | |
| 2 | 100% Ethanol (1.25V) | | | | | | |

**8.** Make sure the Lysis solution has been removed by centrifugation before loading the lysate. Apply 400ul of the lysate/ethanol mixture onto column and centrifuge 1min/10,000g to bind RNA. Repeat until all the lysate/ethanol mixture has been passed through the filter.

**9.** Wash filter with 180ul Wash Solution 1. Centrifuge for 1min/10,000g. Remove flow-through with P1000 pipetman.

**10.** Make 20ul DNase Solution per sample. Mix gently by pipetting (5ul DNase I w/15ul RDD buffer).

*Note: Qiagen DNase stocks are kept at -20°C. Once thawed out, DNase aliquots are kept at 4°C, with the RDD buffer. Thawed DNase stocks are good for up to 6 weeks (write date on side of tube).*

**11.** Pre-warm DNase Solution for 1min at 37°C, then add to filter.

**12.** Incubate at 37°C, 20-30minutes.

**13.** Wash filter:
- a) 180ul Wash Solution 1. Centrifuge for 1min/10,000g.
- b) 180ul Wash Solution 2/3. Centrifuge for 30sec/16,000g.
- c) 180ul Wash Solution 2/3. Centrifuge 30sec/16,000g. Discard flow-through

**14.** Centrifuge 1min/16,000g to dry filter.

**15.** Transfer column to a labeled, clean 1.5ml Micro Elution tube NOT the 2ml tubes provided with the kit. (Use cap of provided 2ml tube to close the column top opening.)

**16.** Add 10ul of Modified Elution Solution (1/10 dilution of Elution buffer: RNase-free 0.01mM EDTA), preheated to 94°C, to the center of the filter. (Make sure to pipet the Elution Solution up and own every time a new tip is used since accurate pipetting of hot liquids can be difficult.)

**17.** Incubate at room temperature, 5minutes. Centrifuge 1min/16,000g.

**18.** Repeat Elution step with an additional 10ul of preheated Modified Elution Solution.

**19.** Cool on ice. Mix RNA by vortexing and spin down briefly.

**20.** Measure RNA concentration using Ribogreen (2.5ul RNA + 2.5ul 200X diluted RG).

**21.** Aliquot RNA for Bioanalyzer (if desired) and store at -80°C.