

LASER CAPTURE MICRODISSECTION H.E. STAINING METHOD



Uniformed Services University
Department of Microbiology and Immunology
4301 Jones Bridge Road
Bethesda, MD 20814

Used by the Laboratory of William C. Gause, Ph.D.

NOTE: STAINING SHOULD BE PERFORMED AS CLOSE AS POSSIBLE TO THE SCHEDULED LCM TRANSFER TIME USING SOLUTION BATHS THAT ARE REPLACED REGULARLY. KEEP IN MIND THAT THE EXTENT OF RNA DEGRADATION IS IN PROPORTION TO THE TIME OF WATER PHASE AFTER FIXATION (5 MINUTES IS THE LIMIT TO OBTAIN GOOD QUALITY RNA). USE DEPC TREATED WATER FOR ALL STEPS IN THIS PROTOCOL.

1. Fix in 70% ethanol for 30 seconds.
2. Wash with purified water by moving slides up and down 10 times for five seconds each.
3. Place in Mayer's hematoxylin for 30 seconds.
4. Wash with purified water by moving slides up and down 10 times for five seconds each.
5. Wash in 70% ethanol for 30 seconds.
6. Wash in 100% ethanol for 30 seconds.
7. Place in Eosin Y for 30 seconds.
8. Wash in 100% ethanol 2 or 3 times for 30 seconds each.
9. Wash in xylene for .5-1 minute. (Optional to wash two times.)
10. Apply to LCM.

LASER CAPTURE MICRODISSECTION RNA ISOLATION PROTOCOL



Uniformed Services University
Department of Microbiology and Immunology
4301 Jones Bridge Road
Bethesda, MD 20814

Used by the Laboratory of William C. Gause, Ph.D.

RNA EXTRACTION

1. Place cap in a 0.5mL Eppendorf Tube containing the following:
 - 200 μ L RNA denaturing buffer (GITC)
 - 1.6 μ L β -mercaptoethanol
 - Invert several times for 2 minutes to digest tissue
2. Remove solution from tube and put it in a new 1.5-mL Eppendorf Tube.
3. Add 20 μ L (0.1X volume) 2M Sodium Acetate (pH 4.0).
4. Add 220 μ L (1X volume) water-saturated phenol (Bottom Half).
5. Add 60 μ L (.3X volume) chloroform-isoamyl alcohol.
6. Vortex vigorously.
7. Place on wet ice for 15 minutes.
8. Centrifuge for 30 minutes at 4°C to separate the organic from the aqueous layer.
9. Transfer upper aqueous layer to new Eppendorf tube.
10. Add 2 μ L of glycogen (10 μ g/ μ L) to help visualize the pellet.
11. Add 200 μ L of cold isopropanol.
12. Place in the -80.0°C freezer for at least 30 minutes. It can remain there over night.
13. Centrifuge for 30 minutes at 4°C with the caps' hinges pointing outward in order to better predict pellet location.
14. Remove the majority of the supernatant with a 1000 μ L, being careful not to disturb the pellet. Remove the rest of the supernatant with a smaller pipet tip to minimize disruption of the RNA.
15. Wash by adding 70% ethanol (400 μ L) and centrifuging for 5 minutes at 4°C.
16. Remove supernatant as described above.
17. Air dry on ice to get rid of excess alcohol and store in -80.0°C freezer.

DNASE

1. Add the following to the RNA pellet in order:
 - 15 μ L DEPC water
 - 1 μ L RNase inhibitor
 - 2 μ L 10X DNase buffer
 - 2 μ L of 10U/ μ L DNase
2. Incubate at 37°C for 2 hours.

RE-EXTRACTION OF RNA

1. Take the tube from DNase step and add the following in order:
 - 2 μ L of NaOAc
 - 22 μ L Phenol
 - 6 μ L Chloroform-isoamyl alcohol
2. Place on ice for 15 minutes.
3. Centrifuge 10 minutes at 4°C.
4. Transfer upper layer to a new tube.
5. Continue with RNA extraction from step 10.

LASER CAPTURE MICRODISSECTION RT-PCR



Uniformed Services University
Department of Microbiology and Immunology
4301 Jones Bridge Road
Bethesda, MD 20814

Used by the Laboratory of William C. Gause, Ph.D.

1. In a tube, add the RNA and water to equal 26 μ L.
2. Add 10 μ L of 5X RT buffer.
3. Add 5 μ L dNTP.
4. Add 4 μ L of 0.1M DTT
5. Add 4 μ L random primers.
6. Add 1 μ L RNasin. The total volume in the tube should be 50 μ L.
7. Incubate for 5 minutes at 70°C.
8. Incubate for 5 minutes at 4°C.
9. Add 2 μ L of Superscript II.
10. Heat for 60 minutes at 37°C.
11. Heat for 5 minutes at 90°C.
12. Incubate for 5 minutes at 4°C.
13. Spin plate down.
14. Store cDNA in -20°C freezer until needed.