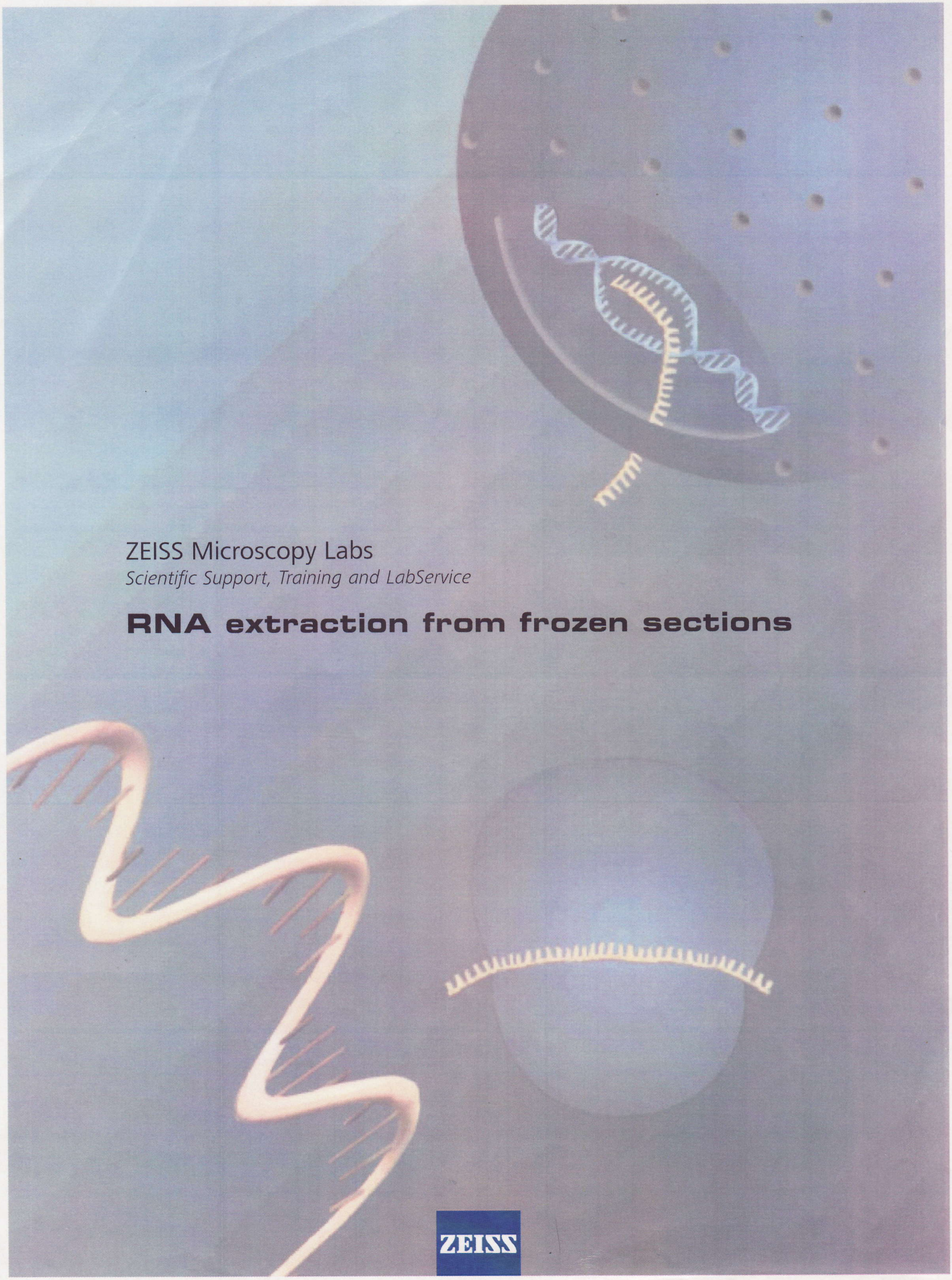

ZEISS Microscopy Labs

Scientific Support, Training and LabService

RNA extraction from frozen sections

ZEISS

Some helpful tips before starting:

- To find additional information around RNA work - especially for less experienced users - please consult our **"LCM Protocols - RNA Handling"** which is available on request.
- To prepare sections we recommend MembraneSlide 1.0 PEN (Order No. 415190-9041-000). To ensure RNase-free conditions, incubate MembraneSlides in dry heat at 180°C for 4 hours to completely inactivate any RNases.
- MembraneSlides Nuclease Free - MembraneSlide NF 1.0 PEN (Order no. 415190-9081-000) are certified to be free of DNase, RNase and human DNA. Treatments to remove nucleases are therefore not necessary when using these slides.
- MembraneSlide 1.0 PET (Order No. 415190-9051-000) may be applicable if weak fluorescence must be detected. For other special applications please inquire.
- For collecting microdissected samples we recommend the special AdhesiveCaps:
  AdhesiveCap 500 opaque (Order No. 415190-9201-000) or
  AdhesiveCap 500 clear (Order No. 415190-9211-000).

Note: To allow subsequent cutting and lifting a coverslip and standard mounting medium must not be applied! Freezing media like OCT or similar may be used for sectioning but should be kept to a minimum and have to be removed before laser cutting.

Microtome cutting and Cresyl Violet staining

The following procedure is recommended especially for RNase-rich tissue and therefore only RNase-free materials must be used and all liquids should be kept on ice.

- For optimal protection of RNA take a pre-cooled MembraneSlide (best inside the cryostat chamber at about -20°C) and touch the backside of the slide with your finger (wear gloves!) to warm only the region for placing the section on the membrane.
- Now transfer the frozen section from the blade to the slide by touching with the warmed area.
- Dry in the cryostat for 2-3 min at about -20°C. Longer drying can increase the adhesion of weakly attaching tissue sections.
- Subsequently incubate the section in ice-cold 70% ethanol for 2-3 min to reduce RNase activity by dehydration.

Note: If excess OCT or another tissue freezing medium was used, an additional washing step in ice-cold RNase-free water (1-2 min) has to be performed after this ethanol step. Dip the slide 5-6 times into the water to enhance the washing effect.

- Now incubate the section in ice-cold CV-staining solution (see below) for 30 sec to 2 min.
- Remove excess stain from the slide on an absorbant surface, then dip few times into ice-cold 70% ethanol for short washing
- Complete washing and dehydration by few dips into ice-cold 100% ethanol
- Finally air-dry at room temperature for 1-2 minutes
- After drying slides can be used immediately for LCM or may be stored at -80°C for some days.

Note: The slides should be frozen and thawed in an airtight container (e.g., two slides back-to-back in a 50 ml Falcon-Tube). To avoid condensation of moisture on the tissue do not open the container before slides are warmed up again to ambient temperature. When sections are completely dry, RNases are inhibited and RNA is remarkably stable for hours.

Preparation of CV-Staining Solution

Dissolve solid Cresyl Violet Acetate (e.g., Aldrich cat #86,098-0) at a concentration of 1% (w/v) in 50% Ethanol. Apply agitation/stirring for several hours to overnight at room temperature for thorough dissolving. Some unsolubilized powder is normal. Filter the staining solution before use.

Another possibility for Cresyl Violet staining is using the LCM Staining Kit from Ambion (#1935). We strongly recommend to omit the final xylene-step of its instruction manual because xylene makes the tissue very brittle and reduces the adhesion of the section to the PEN-membrane.

Applying components of the QIAGEN RNeasy® Micro Kit (Order No. 74004)

1. Add 350 µl Buffer RLT containing ß-Mercaptoethanol to the tube with the LCM ele ments in the AdhesiveCap, close the cap and incubate in an "upside-down" position for 30 min. Please do not use any water bath for the incubation. Thorough lysis is essential for good RNA yield.

   Note: ß-Mercaptoethanol (ß-ME) must be added to Buffer RLT before use. Add 10 µl ß-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT is stable at room temperature for 1 month after addition of ß-ME.

2. Spin down the lysate in a microcentrifuge for 5 minutes (13400 rcf; e.g. Eppendorf 5415D: 12000 rpm).

   Note: Samples can now be stored at -80°C for later use or purified immediately following the original protocol of the QIAGEN RNeasy® Micro Kit (Handbook 04/2003).

3. To continue with the isolation transfer the lysate to a RNase free 1.5 ml micro-centrifuge tube.

4. Now switch to step 5 of the QIAGEN protocol "Total RNA Isolation from Micro-dissected Cryosections" (RNeasy® Micro Handbook 04/2003, pp20).

5. "Add 1 Volume (350 µl) of 70% ethanol to the homogenized lysate, and mix well by pipetting. Do not centrifuge. Continue immediately with step 6."

   Note: All further steps (6-14) of the QIAGEN protocol remain unchanged and should be performed step by step as listed there.
   Please consider also the comments and tips of the QIAGEN RNeasy® manual, especially the section: "Things to do before starting"

- The final RNA solution (12 µl) may be stored at -20°C or used directly for reverse transcription.

Note: Quality control by direct analysis like the Agilent Bioanalyzer (RNA 6000 Pico LabChip® Kit) is limited to concentrations above 50 pg/µl and may only be possible with large microdissected samples (some 2 mm^2 of collected areas from tissue sections of 5-10 µm thickness).

We normally use 5 to 10 µl of the final RNA solution as template in a RT-reaction of 20 µl