ExpressArt LCM RNAready

LCM RNA isolation kit

Catalogue No. 9001-A100

for 100 RNA isolations from laser microdissected samples

This protocol provides the required laboratory procedures

For additional information about ExpressArt technology and products see
http://www.amp-tec.com/products
» more information

Kit Contents

Box I	Volume per RNA isolation	Kit contents for 100 isolations
Lysis Buffer (LB)	350 µl	55 ml
DeS Buffer	130 µl	15 ml
Wash Buffer 1 (WB 1): add 35.0 ml ethanol (100%) to 70.0 ml concentrate of WB 1	1 ml	70 ml concentrate
Wash Buffer (WB 2): add 140.0 ml ethanol (96-100%) to 35.0 ml concentrate of WB 2	1.5 ml	35 ml concentrate
RNase-free water	30 µl	5 ml
Spin columns	1 piece	100 pieces

Box II	Volume per RNA isolation	Kit contents for 100 isolations
N-Carrier	1.0 µl	100 µl
NucleoGuard	3.5 µl	600 µl
RNase-Free DNase	45 µl (working solution)	powder
DiB, Dilution buffer for DNase	39.13 µl	2x 2 ml

Please note, these reagents are not included in the kit
Ethanol, abs.
β-Mercapto-Ethanol

Before you start:

1) **Dissolve lyophilized DNase enzyme in 600 µl RNase-free water.**
   Unused Aliquots are stored at -20°C.
2) **Prepare "DNase working solution" (51 isolations)**
   Add 0.3 ml DNase to 2.0 ml Dilution Buffer (DiB)
   Unused Aliquots are stored at -20°C.
3) **Prepare supplemented Lysis Buffer (LB+) for 25 samples: 350 µl per sample.**
   Use supplemented buffer within one day.

   Preparation of 9.0 ml of LB+
   8.8 ml of Lysis Buffer (LB)
   add 90 µl β-Mercapto-Ethanol. Dispense in a fume hood and wear appropriate protective clothing.
   add 90 µl NucleoGuard, and
   add 25 µl N-Carrier
   N-Carrier is a short hairpin RNA (length ~100 nt) with no activity as primer or as template and does not interfere with downstream applications, including RNA Quality Control with the Agilent Bioanalyzer, ExpressArt mRNA amplification and RT-qPCR.
   NucleoGuard is a universal inhibitor of all RNases and nucleases. It is a low molecular weight chemical that acts nucleic acid analogue (competitive inhibitor) and is safely removed by spin-column clean-up.
4) **Preheat RNase-free water** in thermoblock to 95°C

PROCEDURE

A. Lysis step

A1.Laser microdissected samples

After completion of microdissection, the dry samples are lysed immediately by adding 350 µl of supplemented LB+.
Invert the tubes and incubate for at least 60 min at room temperature, **without agitation**. Proceed to step B.

A2. Harvested cells

Pellet the appropriate number of cells (use a maximum of 100,000 cells; there is no real lower limit, since all samples contain 100 ng of the N-Carrier RNA; we have used numbers as low as ~100 cells) by centrifugation for 5 min at 300 x g in a centrifuge tube (not supplied). Carefully remove all supernatant by aspiration.
Note: Incomplete removal of cell-culture medium will dilute the lysate, may inhibit lysis and affects the conditions for binding of RNA to the column matrix. Both effects may reduce RNA yields.
Loosen the cell pellet thoroughly by flicking the tube. Add 350 µl of supplemented LB+ and vortex or pipette to mix.
Homogenize by vortexing for 1 min. Proceed to step B.

B. Spin column purification

1. **Transfer lysates in 2 ml reaction tubes.**
   Add 20 µl water and 90 µl Demodification Solution (DeS) per 350 µl Lysis Buffer (LB+). Mix by pipetting, then add 575 µl EtOH abs (1.25x Vol.)
   Mix again by pipetting (Volume = 1035 µl)

2. **Transfer an aliquot of 700 µl of this mixture on column**
   Centrifuge: 30 sec @ 10,000 rpm & discard flow-through
   Transfer the remaining mixture on column
   Centrifuge: 30 sec @ 10,000 rpm & discard flow-through

3. **Add 500 µl Wash Buffer 1 (WB 1)**
   Centrifuge: 30 sec @ 10,000 rpm & discard flow-through

4. **Plus 500 µl Wash Buffer 2 (WB 2)**
   Centrifuge: 30 sec @ 10,000 rpm & discard flow-through

5. **DNA-Digestion:**
   Transfer 45 µl of the “DNase Working solution” on column
   Incubate: 15 min @ RT.

6. Plus 500 µl **Wash Buffer 1 (WB 1)** Incubate: 1 min
   Centrifuge: 30 sec @ 10,000 rpm & discard flow-through

7. Plus 500 µl **Wash Buffer 2 (WB 2)**
   Centrifuge: 30 sec @ 10,000 rpm & discard flow-through

8. Plus 500 µl **Wash Buffer 2 (WB 2)**
   Centrifuge: 30 sec @ 10,000 rpm & discard flow-through

9. Add 500 µl 80% EtOH (at room temperature)
   Centrifuge: 30 sec @ 10,000 rpm & discard flow-through

10. Re-insert the column in the same collection tube, and centrifuge for 1 min@maximum speed to get rid of residual salt on the spin column matrix.

11. **Elution:** Transfer column in fresh 1.5 ml reaction tube and add **30 µl RNase-free water** (preheated at 95℃)
    Incubate: 2 min and centrifuge for 1 min@maximum speed

12. Reapply the eluate on the column
    Incubate: 2 min and centrifuge for 1 min@maximum speed

RNA is in a total volume of ~30 µl.
Use RNA immediately or store at -80℃.

Note for subsequent mRNA amplification

If required, the volume of the eluted RNA can be reduced by limited concentration in a SpeedVac (avoid complete drying!).

Further note:
A modified kit protocol can be used for mRNA amplification with the ExpressArt Nano kit:
A larger volume of the isolated RNA (~10 µl) can be used, if water is omitted in **Mix 1** and **Mix 2**.