ExpressArt LBR RNAready

RNA isolation kit
for solid tissues or for Bacteria
in combination with mechanical lysis (beads)

Catalogue No. 9005-A100

for 100 RNA isolations

including on-column DNase digestion

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**
1st Lysis Buffer LBR-1 (no detergent)	**350 µl**	**40 ml**
2nd Lysis Buffer LBR (with detergent)	**300 µl**	**30 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**
NucleoGuard	**6.5 µl**	**650 µl**
RNase-Free DNase	**45 µl (working solution)**	**powder**
DiB, Dilution buffer for DNase	**39.13 µl**	**2x 2 ml**

Please note, these consumables are not included in the kit
For solid tissues: Precellys CKM tubes (www.peqlab.com Cat.-No. 91-PCS-CKM)
For bacterial samples: Precellys VK01 tubes (www.peqlab.com Cat.-No. 91-PCS-VK01)
Ethanol, abs.
β-Mercapto-ethanol

Required equipment
Precellys homogeniser (www.peqlab.com) or FastPrep homogeniser (www.qbiogene.com)
Thermomixer (www.eppendorf.com) or Thermoshaker (www.peqlab.com)

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 Buffers**
   Use supplemented buffer within one day.

   Preparation of 10.0 ml LBR-1+ and LBR+
   10 ml of Lysis Buffer, add 100 µl each: β-Mercapto-Ethanol and NucleoGuard.
   Dispense in a fume hood and wear appropriate protective clothing.
4) **Preheat RNase-free water in thermoblock to 95°C**

PROCEDURE

A. Lysis step

A1. Solid tissues

A1.1 **Use Precellys CKM tube (contains a mixture of 1.4 & 2.8mm ceramic Zirkonium beads) and add 350 µl 1st Lysis Buffer LBR-1+**

A1.2 **Transfer fresh/frozen biopsy (up to ~50 mg) to the CKM tube.**
Homogenise for 2x 30sec@6.5K (15 sec Pause) Maybe, some optimisation is required.

A1.3 **Add 300 µl 2nd Lysis Buffer LBR+**

A1.4 **Incubate for 15 min@RT in Thermomixer@1000 rpm.**

A1.5 **Transfer ~600 µl supernatant to fresh 1.5ml tube, spin for 5 min@max speed.**

A1.6 **Transfer ~580 µl supernatant to fresh 2ml tube**
Per 580 µl of transferred lysate: Add 150 µl water and 910 µl EtOH abs and mix.

Proceed to step B.

A2. Bacterial samples

A2.1 **Use Precellys VK01 tube (contains 0.1 mm glass beads)**
and add 350 µl 1st Lysis Buffer LBR-1+

A2.2 **Transfer fresh/frozen sample (up to 50 mg) to the VK01 tube.**
Homogenise for 2x 20sec@5.5K (5 sec Pause) Maybe, some optimisation is required.

A2.3 **Add 300 µl 2nd Lysis Buffer LBR+**

A2.4 **Incubate for 15 min@RT in Thermomixer@1000 rpm.**

A2.5 **Transfer ~600 µl supernatant to fresh 1.5ml tube, spin for 5 min@max speed.**

A2.6 **Transfer ~580 µl supernatant to fresh 2ml tube**
Per 580 µl of transferred lysate: Add 150 µl water and 910 µl EtOH abs and mix.

Proceed to step B.

B. Spin column purification

B1 **Transfer an aliquot of 700 µl of the 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

B2 **Add 500 µl Wash Buffer 1 (WB 1)**
Centrifuge: 30 sec @ 10,000 rpm & discard flow-through

B3 **Plus 500 µl Wash Buffer 2 (WB 2)**
Centrifuge: 30 sec @ 10,000 rpm & discard flow-through

B4 **DNA-Digestion:**
Transfer 45 µl of the "DNase Working solution" on column
Incubate: 15 min @ RT.

B5 **Plus 500 µl Wash Buffer 1 (WB 1) Incubate: 1 min**
Centrifuge: 30 sec @ 10,000 rpm & discard flow-through

B6 **Plus 500 µl Wash Buffer 2 (WB 2)**
Centrifuge: 30 sec @ 10,000 rpm & discard flow-through

B7 **Plus 500 µl Wash Buffer 2 (WB 2)**
Centrifuge: 30 sec @ 10,000 rpm & discard flow-through

B8 **Add 500 µl 80% EtOH (at room temperature)**
Centrifuge: 30 sec @ 10,000 rpm & discard flow-through

B9 **Re-insert the column in the same collection tube,**
centrifuge for 1 min@maximum speed to get rid any residual liquid

B10 **Elution: Transfer column in fresh 1.5 ml reaction tube and**
add 30 µl RNase-free water (preheated at 95°C)
Incubate: 2 min and centrifuge for 1 min@maximum speed

B11 **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°C.

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 mRNA amplification kits:

A larger volume of the isolated RNA (~10 µl) can be used, if water is omitted in Mix 1 and Mix 2.

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