ExpressArt FFPE RNAready

FFPE RNA isolation kit

Catalogue No. 9000-A100

for 100 RNA isolations from archival paraffin 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)	500 µl	55 ml
Demodification Solution (DeS)	130 µl	15 ml
Wash Buffer 1 (WB 1) Add 35.0 ml ethanol (96-100%) 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	5 µl	0.6 ml
Proteinase K, PCR Grade*	30 µl	powder
RNase-Free DNase**	45 µl (working solution)	powder
DiB, Dilution buffer for DNase	39.13 µl	2x 2 ml

*** Dissolve proteinase K (powder) in 4 ml water (25 mg/ml)**; store at -20°C.

****Prepare "DNase working solution"**
First step:
Dissolve DNase (powder) in 600 µl water; store at -20°C.
Second step:
DNase working solution (prepare according to your needs)
The example is for 51 isolations:
Mix 0.3 ml DNase + 2.0 ml Dilution Buffer (DiB)
Unused Aliquots are stored at -20°C

Please note, these reagents are not included in the kit
Xylene
Ethanol, abs.

Required instrument
Thermomixer (Eppendorf # 5350 000.013 or "Thriller" from PEQLAB #91-7010) or equivalent

Tissue samples
Up to 5 FFPE slides (10 µm thick with appr. 1-2 cm^2 of tissue) per isolation

1. Deparaffinisation (in Thermomixer with 1000 rpm)

Insert paraffin sections in reaction tube
Add 1.5 ml xylene and incubate for 20 min@37℃
Then centrifuge for 3 min @ 16'000 g and remove xylene (important!)
Repeat:
Add 1.5 ml xylene and incubate for 20 min@37℃
Then centrifuge for 3 min @ 16'000 g and remove xylene (important!)

Then add 1 ml EtOH abs
"Flick tubes" (to dislodge pellet)
Centrifuge for 3 min @ 16'000 g and remove ethanol
Dry at 37℃ (can take some time – but pellet has to be dry – not "**sticky"!**)

Dissolve dry pellet in
500 µl **Lysis Buffer (LB)** + 30 µl Proteinase K (25 mg/ml) + 5 µl NucleoGuard

2. Lysis: Incubation in Thermomixer (1000 rpm) for 3 h @ 55℃

3. Demodification: Add 130 µl **Demodification Solution (DeS)** (to 535 µl lysate)
Vortex, then 20 min @ 80℃
Chill on ice (3 min)

4. RNA Purification
Before you start: Put **RNase-free water** in thermoblock for heating to 95℃

Centrifuge lysate: 3 min @ 13'000 rpm
Transfer 600 µl of the supernatant in 2 ml reaction tube
Add 750 µl EtOH abs (1.25x Vol.)
Mix by pipetting (Volume = 1350 µl)

Transfer aliquot of 700 µl of the EtOH-supplemented lysate on column
Centrifuge: 30 sec @ 10'000 rpm & discard flow-through
Transfer the remaining lysate on column & discard flow-through
Add 500 µl **Wash Buffer 1 (WB 1)**
Centrifuge: 30 sec @ 10'000 rpm & discard flow-through
Plus 500 µl **Wash Buffer 2 (WB 2)**
Centrifuge: 30 sec @ 10'000 rpm & discard flow-through
Centrifuge: 1 min @ Max

4.1. DNA-Digestion: Transfer 45 µl of the “DNase Working solution” on column
Incubate: 15 min @ R.T.
Plus 500 µl **Wash Buffer 1 (WB 1)** Incubate: 1 min
Centrifuge: 30 sec @ 10’000 rpm & discard flow-through
Plus 500 µl **Wash Buffer 2 (WB 2)**
Centrifuge: 30 sec @ 10’000 rpm & discard flow-through
Plus 500 µl **Wash Buffer 2 (WB 2)**
Centrifuge: 30 sec @ 10’000 rpm & discard flow-through
Centrifuge: 1 min @ Maximum speed

4.2. Elution: Transfer column in fresh 1.5 ml reaction tube
Add 30 µl **RNase-free water** (preheated at 95℃)
Incubate: 2 min
Centrifuge: 1 min @ Maximum speed
Reapply the eluate on column
Incubate: 2 min
Centrifuge: 1 min @ Max
Transfer eluate to fresh tube for storage or for further processing (avoid risk: lid can get contaminated during entrifugation).

RNA is recovered in a total volume of ~30 µl

Expected yield ~10-30 µg RNA (~2-6 µg per slide)

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