ExpressArt Mag FFPE Clear RNAready kit

FFPE RNA isolation kit

Catalogue No. 9011-A100

for 100 RNA isolations from archival paraffin samples

with Magnetic Beads for RNA isolation

No separate paraffin removal step !

This protocol provides the required laboratory procedures

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

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Kit Contents

Box I	**Volume per RNA isolation**	**Kit contents for 100 isolations**
Lysis Buffer (LB)	**870 µl**	**87 ml**
FFPE Clear a formulation of ***non-toxic, non-odorous*** organic solvents	**500 µl**	**55 ml**
Demodification Solution (DeS)	**130 µl**	**15 ml**
RNase-free water	**50 µl**	**5 ml**

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**
Magnetic Beads Stock	**100 µl**	**10 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

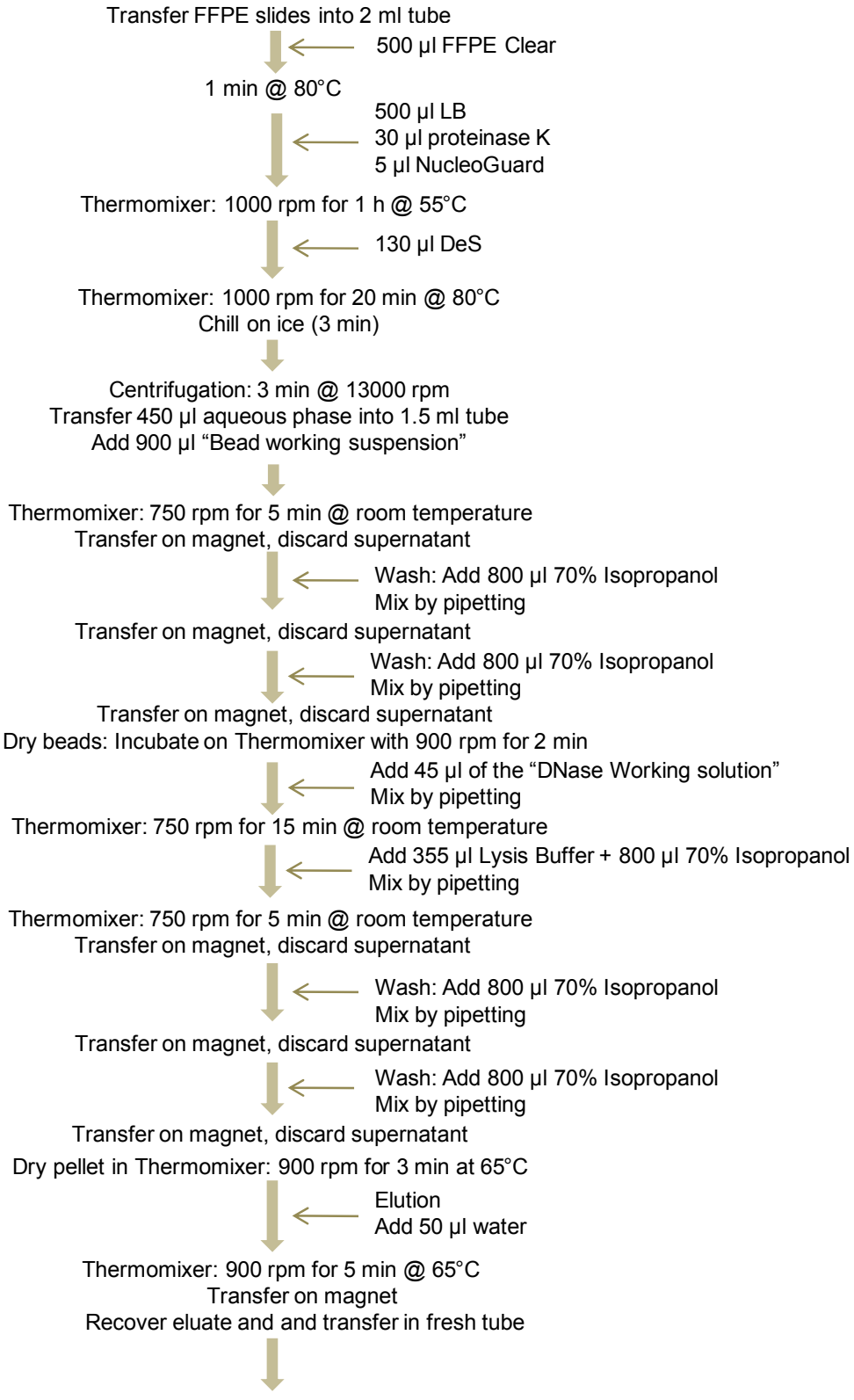
Please note, these reagents are not included in the kit
Isopropanol

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

Tissue samples
Please note:
1) to avoid negative surface effects (contact to air), do not use the first 2 slides for preparation of nucleic acids.
2) no need for immediate processing of slides,
freezing and short-term storage (up to 3 days) at -80°C is even advantageous:
there is no negative effect on RNA quality; frequently, RNA yields will increase significantly

Up to 5 FFPE slides (10 µm thick with appr. 1-2 cm^2 of tissue) per isolation can be used in the lysis procedure

Work Flow
RNA isolation from FFPE samples using the ExpressArt Mag FFPE Clear RNAready kit



Total Time: ~2 h

1. Sample preparation
Insert paraffin sections in reaction tube
Immediately (no prior paraffin removal required) add 500 µl **FFPE Clear**
(Note: For immediate processing, it can be more convenient to prepare tubes with FFPE Clear, and transfer paraffin sections directly into the solvent, avoiding ”electrostatic problems”)
Heat for 1 min @ 80°C.
Add 500 µl **Lysis Buffer (LB)** + 30 µl Proteinase K (25 mg/ml) + 5 µl NucleoGuard

2. Lysis: Incubation in Thermomixer (1000 rpm) for 1 h @ 55°C

3. Demodification: Add 130 µl **Demodification Solution (DeS)** (to 535 µl lysate)
Vortex, then incubation in Thermomixer (1000 rpm) for 20 min @ 80°C
Chill on ice (3 min)

4. RNA Purification
Before you start, prepare ”bead suspension”
For 1 sample: Transfer 100 µl of Magnetic Beads Stock into 1.5 ml tube, place on magnet, discard liquid and resuspend pellet in 900 µl 70% isopropanol.
For multiple samples, use two suspension steps
Example for **10 samples: Step 1:** Transfer 1 ml of Magnetic Beads Stock into 1.5 ml tube, place on magnet, discard liquid and resuspend pellet in 1 ml of 70% isopropanol by pipetting.
Step 2: Transfer into 15 ml tube, add 8 ml 70% isopropanol and mix thoroughly.

4.1. Recovery of the aqueous lysate
Centrifuge lysate (from step 3): 3 min @ 13’000 rpm
This results in three zones: pellet at the bottom (debris), **aqueous phase** with the lysate in the middle and organic supernatant at the top.

4.2. RNA binding to beads
Transfer 450 µl of the **aqueous phase** (avoid transfer of the organic supernatant) in 1.5 ml reaction tube
Mix 450 µl of the aqueous lysate with 900 µl bead suspension by pipetting (= 1350 µl)
Incubate on Thermomixer (750 rpm) for 5 min @ room temperature
Transfer on magnet, discard supernatant

4.3. First washing steps
Wash 2x with 800 µl 70% Isopropanol: Mix by pipetting, place on magnet, remove liquid
For optional DNA-Digestion proceed with steps 4.4 to 4.6, or go directly to step 4.7.

4.4. DNA-Digestion
Incubate on Thermomixer (900 rpm) for 2 min @ room temperature to dry the beads.
Add 45 µl of the “DNase Working solution” to beads, mix by pipetting.
Incubate on Thermomixer (750 rpm) for 15 min @ room temperature

4.5. DNase inactivation and RNA recovery
Add 355 µl Lysis Buffer + 800 µl 70% Isopropanol and mix by pipetting
Incubate on Thermomixer (750 rpm) for 5 min @ room temperature
Transfer on magnet, discard supernatant

4.6. Second washing steps

Wash 2x with 800 µl 70% Isopropanol: Mix by pipetting, place on magnet, remove liquid

4.7. Elution

Incubate on Thermomixer (900 rpm) for 3 min at 65°C to dry the beads completely
Add 50 µl water, mix by pipetting and make sure to soak the beads completely
Incubate on Thermomixer (900 rpm) for 5 min @ 65°C
Transfer on magnet and transfer supernatant in fresh tube

RNA is recovered in ~45 µl.
Expected yield 2 - 6 µg RNA per FFPE slide

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