XTRAKT FFPE Kit / Manual

For

manual extraction of

RNA, miRNA and/or DNA

from

Formalin Fixed Paraffin Embedded

(FFPE) tissue samples

Catalog No. # XTK2.0-96

For research use only. Not intended for diagnostic purposes.

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1. MATERIALS

1.1 Kit Contents

• Lysepuffer-Paraffin [Lysis Buffer]	20 ml
• MagiX-Beads	6,5 ml
• MagiX-RNA [MagiX-RNA Binding Buffer]	100 ml
• Waschpuffer 1[Wash Buffer 1] add 31,5 ml ethanol before first-time use	105 ml
• Waschpuffer 2 [Wash Buffer 2] add 48 ml ethanol before first-time use	60 ml
• Waschpuffer 3 [Wash Buffer 3]	105 ml
• Elutionspuffer [Elution Buffer]	15 ml

*all RNA buffers can also be used for DNA extraction (Roedel et al., Int. J. Cancer 2015: 136, 278–288

1.2 Equipment

2 Thermomixers (suitable for 1.5 ml tubes, one with cooling function)
Vortexer
Microcentrifuge
Magnetic separation rack (e.g. DynaMagTM-2, Life Technologies, Cat. No. 12321D)
Adjustable Pipettes

1.3 Consumables

1.5 ml reaction tubes RNase/DNase-free (e.g. Eppendorf, Cat.No.# 0030 120.086)
Sterile RNase free filter tips
Disposable gloves
Reagent for decontamiation (e.g. DNAZap, Ambion, Cat.No.# 9890, or equivalent)

1.4 Additional Reagents (not supplied)

Nuclease-free water
Ethanol ≥ 99.5%
Proteinase K (Roche, Cat.No.# 03115852001, store at 4°C)
(dissolve 250 mg Proteinase K in 12,5 mL 10 mM Tris pH8, store aliquots at -20°C)
DNase I (RNase-free) (Cat.No.# AM2222 or AM2224, Ambion, store at -20°C)

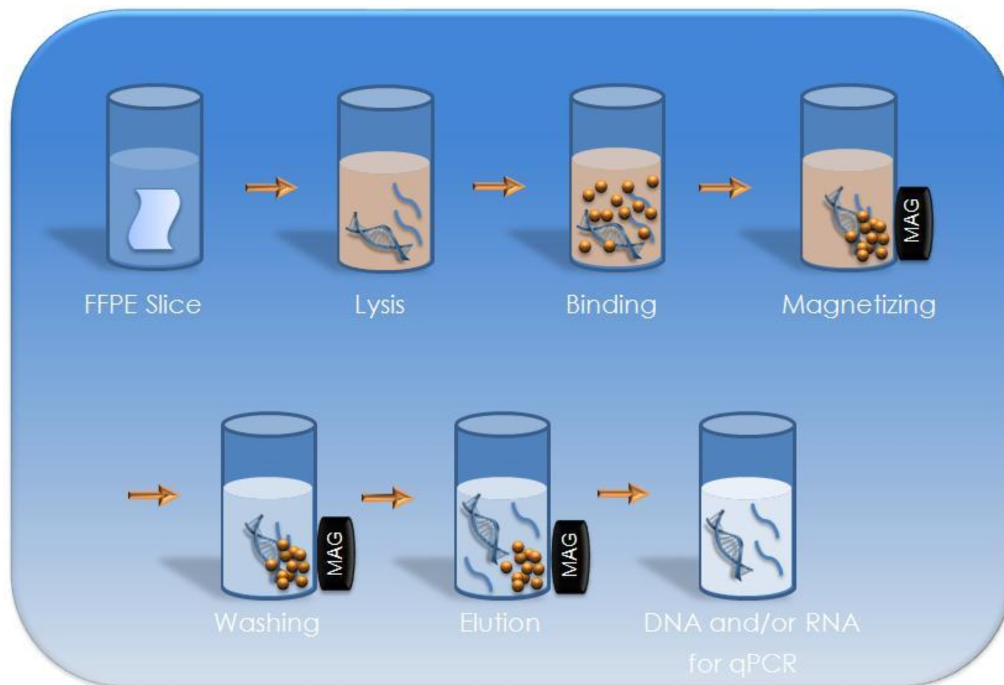
Store all reagents at room temperature (unless otherwise noted).
Wash Buffer 1 and 2 are stable for 3 months at room temperature after the addition of Ethanol.

2. METHODEN ÜBERBLICK

The XTRAKT FFPE Kit is designed for the extraction and purification of RNA, miRNA and / or DNA from formalin fixed, paraffin-embedded (FFPE) tissue samples.

The newly developed method combines proprietary germanium coated paramagnetic beads with specalized buffer systems. Highly efficient binding conditions guarantee the recovery of nucleic acids with up to 4 fold higher yield compared to other suppliers in just 2,5 h (including a 30 min DNase step for removal of DNA).

Between 1and 3 FFPE sections with maximum thickness of 10 µm can be extracted in one tube. No Deparaffinization step with xylene is required.



Schematic representation of the **STRATIFYER** purification method with integrated deparaffinization step. The affinity of the bead surface to nucleic acids is modulated with appropriate buffers.

3. PROCEDURE

Before starting:
Add ethanol (≥ 99.5 %) to Wash Buffers 1 and 2

3.1 Deparaffinization

Attention: no deparaffinization with xylene is required.

3.2 Purification

1. Centrifuge the tubes with FFPE section(s) for 1 min at ≥ 15,000 x g to collect section(s) at the bottom of the tubes
2. Add 150 µl Lysis Buffer to each tube
3. Incubate at 80°C for 30 min with shaking at 1200 rpm
4. Cool down to 65°C (10 min)
5. Add 50 µl Proteinase K (20 mg/ml)
6. Incubate at 65°C for 30 min with shaking at 1200 rpm
7. Add 800 µl MagiX-RNA Binding Buffer
8. Add 40 µl MagiX-Beads to each tube
   Vortex MagiX-Bead suspension vigorously for 2 min before adding to the sample

   Note: in case of processing more than 3 samples simultaneously, vortex bead suspension briefly after every third sample
9. Incubate at room temperature for 15 min with shaking at 1200 rpm
10. Place the tubes on the magnetic separation rack
11. Invert the tubes on the magnetic separation rack several times to remove all beads from the cap. Wait until all beads have bound to the magnet (at least 10 sec)
12. Aspirate and discard the supernatant while tubes are bound to the magnet
13. Remove the tubes from the magnetic separation rack. Add 850 µl Wash Buffer 1. Cap the tubes and mix by inverting several times
14. Place the tubes on the magnetic separation rack
15. Invert the tubes on the magnetic separation rack several times to remove all beads from the cap. Wait until all beads have bound to the magnet (at least 10 sec)
16. Aspirate and discard the supernatant while tubes are bound to the magnet

17. Remove the tubes from the magnetic separation rack. Add 450 µl Wash Buffer 2. Cap the tubes and mix by inverting several times
18. Place the tubes on the magnetic separation rack
19. Invert the tubes on the magnetic separation rack several times to remove all beads from the cap. Wait until all beads have bound to the magnet (at least 10 sec)
20. Aspirate and discard the supernatant while tubes are bound to the magnet
21. Remove the tubes from the magnetic separation rack. Add 850 µl Wash Buffer 3. Cap the tubes and mix by inverting several times
22. Place the tubes on the magnetic separation rack
23. Invert the tubes on the magnetic separation rack several times to remove all beads from the cap. Wait until all beads have bound to the magnet (at least 10 sec)
24. Aspirate and discard the supernatant while tubes are bound to the magnet
25. Briefly centrifuge (5 sec.) the sample to collect the rest of Wash Buffer 3 at the bottom of the tubes
26. Place the tubes on the magnetic separation rack
27. Aspirate and discard the residues of the supernatant while tubes are bound to the magnet
28. Add 100 µl Elution Buffer
29. Incubate at 70°C for15 min with shaking at1200 rpm in a thermomixer
30. Place the tubes on the magnetic separation rack. Transfer the eluate into a fresh tube

3.3 DNAse I treatment

To obtain pure RNA and miRNA add 10 µl 10 x DNAse Buffer and 2 µl DNAse I to the eluate
(alternatively add 5 µl 10 x DNAse Buffer and 1 µl DNAse I to 50 µL of the eluate. The rest of the eluate contains DNA as well).

Incubate 30 min at 37°C without shaking

4. STORAGE OF PURIFIED ELUATE

The purified eluates should be directly stored at -80°C until use. Depending on the application, aliquoting the eluates to avoid multiple freeze and thaw cycles should be considered.
The purified eluates can be measured by photometric determination or gel electrophoresis and can be analyzed by qRT-PCR, nCounter and NGS.