










Instructions for Use**RealLine FFPET DNA Extraction Mag****EXTRACTION KIT FOR DNA FROM FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUE ON MAGNETIC BEADS BASIS**

For research use only.

RealLine FFPET DNA Extraction Mag	MED32501	12 Tests
	MED32503	48 Tests
valid from	October 2019	

RealLine FFPET DNA Extraction Mag

Explanation of symbols used in labelling

	For research use only
	Batch code
	Catalogue number
	Contains sufficient for <n> tests
	Use-by-date
	Temperature limit
	Consult instructions for use
	Keep away from sunlight
	Manufacturer



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RealLine FFPET DNA Extraction Mag

EXTRACTION KIT FOR DNA FROM FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUE SPECIMENS

For research use only

1. INTENDED USE

The **RealLine FFPET DNA Extraction Mag** kit is designed for DNA extraction from formalin-fixed and paraffin-embedded (FFPE) tissue specimens. The kit can be used also for the DNA extraction from fresh-frozen tissue.

DNA purified with the kit can be used in the polymerase chain reaction (PCR) including real-time PCR.

The kit is designed for DNA isolation from 12 samples. The kit for 12 samples has sufficient reagents to conduct 2 independent experiments, each for the isolation of 6 samples.

2. PRINCIPLE OF THE METHOD

Using **RealLine FFPET DNA Extraction Mag** kit FFPE tissue is simultaneously melted, lysed and de-cross-linked in a specially formulated solution. This kit does not require de-waxing with xylene and lengthy digestion with Proteinase K. After FFPE tissue lysis DNA is precipitated onto magnetic particles, then washed and eluted ready for PCR.

Magnetic stand is recommended for convenience of work with a kit.

3. KIT COMPONENTS

	MED32501	MED32503
Solution A*	1 vial, 2.5 ml	4 x MED32501
Solution B	1 vial, 250 µl	
Lysis Reagent	2 vials, 4 ml each;	
Solution for DNA Precipitation	1 vial, 12 ml each;	
Wash Solution No.1	1 vial 8 ml each;	
Wash Solution No.2	1 vial, 5 ml each;	
Specimen Diluent	3 vials, 3 ml each;	
Sorbent (suspension of magnetic particles)	1 vial, 250 µl;	

* contains sodium hydroxide with detergents. See section 4 for safety information

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4. WARNING AND PRECAUTIONS

- ☞ For In vitro use only.
- ☞ The kits must be used by skilled personnel only.
- ☞ Always wear a lab coat, disposable gloves and protective goggles when working with the kit, since all human biological material should be treated as potentially infectious.
- ☞ Solution A contains diluted sodium hydroxide and detergent, which is irritating to eyes and skin. In case of contact with eyes or skin wash with plenty of water and seek medical advice;
- ☞ Avoid microbial and nuclease contamination of reagents when removing aliquots from reagent vials. The use of sterile disposable pipettes and DNase-free pipette tips is recommended.
- ☞ Do not pool reagents from different lots or from different vials of the same lot.
- ☞ Dispose unused reagents and waste in accordance with country, federal, state and local regulations.
- ☞ Do not use the kit after the expiration date.
- ☞ Treat all disposable materials with disinfectant before utilization.

5. ADDITIONAL MATERIALS AND DEVICES REQUIRED

- magnetic tube holder;
- laminar flow hood;
- refrigerator;
- Eppendorf-type microcentrifuge with a maximum rotation speed of at least 13 000 rpm;
- vortex;
- heating block for +37 °C...+95 °C;
- Thermo Shaker;
- variable-volume single-channel pipettes;
- disposable medical non-sterile powder-free gloves and lab coat;
- disposable pipette tips with aerosol barrier;
- biohazard waste container

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6. PREPARATION OF REAGENTS AND SPECIMENS

6.1 Specimens preparation

Formalin-fixed paraffin embedded tissue blocks should be cut on slides 10 µm thick and verified by pathologist. If dissection is required spread 4 - 6 slides on to glass for microscopy and dry overnight. The first and the last control sections are stained with H&E and verified by microscopy. Using control slides mark the area of interest on the remaining 2 - 4 sections. Add 20 - 25 µl of deionised water to the slide and scrape out selected regions using single-use blade. Transfer slide pieces in a 1.5 ml tube.

If dissection is not required place 2 slides (tissue area 25 – 80 mm² per slide) in a 1.5 ml tube.

6.2 Reagent preparation

Before use:

- warm reagents at the room temperature (18 – 25) °C for 30 minutes;
- warm Solution A to (30 - 40) °C and mix thoroughly to dissolve the precipitated material;
- warm Lysis Reagent to (50 - 60) °C and mix thoroughly to dissolve the precipitated material. Shake the vial with Sorbent on Vortex to make homogeneous suspension.
- Add 80 µl of Sorbent suspension into a vial with Lysis Reagent. Mix carefully.

Attention! Once opened, any unused portion of Lysis Reagent with addition of Sorbent should be discarded.

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7. EXTRACTION PROTOCOL

- 7.1 Add **200 µl Solution A** in the tube with FFPE tissue slides, incubate 20 - 30 min at 95 °C. Use tubes with safe-lock or place clean glass plate with weight on top of tubes to prevent caps from opening due to air pressure.
- 7.2 Carefully remove the tube from heating block avoiding cap opening. Cool 10 min at 4 °C in the refrigerator. Spin 30 sec at 10 000 rpm and RT in microcentrifuge.
- 7.3 Carefully transfer solution from under the paraffin layer into the new 1.5 ml tube. Add to the DNA extract **20 µl Solution B**. Mix and spin 5 min at 10,000 rpm and RT to collect mixture at the bottom of the tube.
- 7.4 Carefully transfer the supernatant to a new 1.5 ml tube and add **300 µl Lysis Reagent with Sorbent**. Vortex for 10 - 15 seconds. Place the tubes into Thermo Shaker, and incubate for 10 minutes at 65 °C and 1300 rpm. Spin shortly to collect the drops.
- 7.5 Add **400 µl Solution for DNA Precipitation** in each tube. Vortex for 10 - 15 seconds.
- 7.6 Centrifuge at 13,000 rpm for 5 minutes at RT.
- 7.7 Trying not to shake up the pellet, place the tubes into Magnetic Stand. Using a new tip for each sample, carefully remove the supernatant without disturbing the pellet.
- 7.8 Add **500 µl Wash Solution № 1** in each tube. Vortex vigorously for 10 - 15 seconds. Centrifuge at 13,000 rpm for 2 minutes at RT.
- 7.9 Trying not to shake up the pellet, place the tubes into Magnetic Stand. Using a new tip for each sample, carefully remove the supernatant without disturbing the pellet.
- 7.10 Add **300 µl Wash Solution № 2** to each tube. Vortex vigorously for 10 - 15 seconds. Centrifuge at 13,000 rpm for 2 minutes at RT.
- 7.11 Trying not to shake up a pellet, place the tubes to Magnetic Stand. Using a new tip for each sample, carefully remove the supernatant without disturbing the pellet.
- 7.12 Dry the pellet with open caps for 2-3 minutes at room temperature (18 - 25) °C.
- 7.13 Add **200 µl** (up to 600 µl, if necessary) **Specimen Diluent** to each tube. Vortex vigorously for 10 - 15 seconds. Place the tubes into Thermo Shaker, and incubate for **5 minutes at 65 °C** and 1300 rpm. Then centrifuge for 1 minute at 13,000 rpm and RT.
- 7.14 Samples are ready for PCR. For storage please transfer supernatant to sterile tubes without pipetting magnetic beads.

Isolated DNA can be stored at 2 °C – 8 °C for 24 hours or up to 12 months at -15 °C...-25 °C.

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8. STORAGE AND TRANSPORTATION

- Store the kit at (2 - 8) °C in the manufacturer's packing.
- Transportation at 25 °C for up to 10 days is allowed.
- Do not freeze the kit!
- Do not pool reagents from different lots or from different vials of the same lot.
- Strictly follow the Instruction manual for reliable results.
- Do not use kits with damaged packages. In case of damaged package contact with BIORON Diagnostics GmbH.

Technical Support: techsupport@bioron.de

