





Introduction

The **DNrich** FFPE Tissue Kit provides all of the reagents necessary to extract DNA from a wide variety of Formalin-Fixed Paraffin Embedded tissue sources. DNA purified with this kit is suitable for a variety of applications, including amplification and digestion with restriction endonucleases.

DNrich FFPE Tissue Kit components*

Cat. No. AEFDA -1059	1
G Solution	50 ml
ZR Solution(conc.) **	15 ml
TD Buffer	20 ml
Activator Reagent**	2 ml
VD Buffer	10 ml
SE Buffer	15 ml
Wash Buffer(conc.) **	15 ml
Elution Buffer	3 ml
Column	50
Manual	1

^{* 1.5} ml microcentrifuge tube, **molecular biology grade** water and absolute ethanol are needed but are not included.

Chemical Hazard

Always wear gloves and practice standard safety precautions while using the kit. Do NOT disinfect extraction waste in solutions containing **bleach** or any other form of acid. To clean any items contaminated with the reagent, simply soak in detergent and water to remove all traces of contamination before cleaning with bleach or acidic solutions.

Reminder

- Pre-set heather block at 65°C.
- Prepare **Activator Reagent** immediately prior to use. Prepared Activator Reagent must be kept at 4°C.
- Prepare **Wash Buffer** before first use.
- During DNA extraction, never open the microtube cap outside the laminar hood.

Activator Reagent Preparation

 Add 2 ml of molecular biology grade water to Activator Reagent, and vortex it well.

Note: Mark the check box on the bottle and write the date. **Note:** For the best results, the prepared Activator Reagent should be used immediately. Prepared Activator Reagent can be stored for up to 3 months or 6 months at 4°C and -20°C, respectively.

Wash Buffer and ZR Solution Preparation

 Add 35 ml molecular grade absolute ethanol to Wash Buffer and ZR Solution bottle before first use and mark the check box on the bottle.

Symbols

The following symbols are used in the labeling:

REF	Catalogue number
	Manufacturer
1	Temperature limit
LOT	Batch code
\subseteq	Use-by date
Σ	Contains sufficient for <n> tests</n>
[]i	Consult instructions for use

Unit 1, No 30, Pakestan Street, Shahid Beheshti Avenue, Tehran, Iran Tel: 021-88735747, 021-88754510

Web address: https://azmaelixir.com/

E-mail: info@azmaelixir.com

^{**} Please refer to reminder, Activator Reagent, Wash Buffer and ZR Solution Preparation before using this kit

PROTOCOL

Step 1: Sample Preparation

• Transfer **50 mg** (up to 200 mg) of tissue sample into a 1.5 ml tube.

Step 2: Paraffin Removal

- Add 500 µl G Solution to sample tube and vortex vigorously.
- Incubate at 65°C for 2 minutes.
- Centrifuge at 12000 g for 5 minutes.
- **Decant** the supernatant into waste.
- Repeat step 2.

Step 3: Tissue Washing

- Add 500 µl ZR Solution to sample tube and vortex it.
- Centrifuge at **12000** g for **5** minutes.
- **Decant** the supernatant into waste.
- Note: Repeat this step to extract a pure DNA.

Step 4: Tissue Digestion & Lysis

Note: Refer to reminder before use activator reagent

- Add 400 µl of TD Buffer and 40 µl of Activator Reagent to the sample tube and vortex vigorously.
- Incubate at 65°C for 30 minutes.

Note: During incubation time, vortex the sample tube every 10 minutes.

- Incubate at 85°C for 10 minutes and vortex every 5 minutes.
- Add 200 µl of VD Buffer to sample tube and vortex vigorously and then keep at RT for 5 minutes.
- Centrifuge at 12000 g for 10 minutes.

Note: Do not disturb the phases.

- Carefully transfer about **300µl of supernatant** to a new 1.5 ml tube.
- Add 300μl of SE Buffer to the tube, invert for 5 times and keep at room temperature for 3 minutes and then transfer all the sample to a spin column.
- Centrifuge at 2000 g for 2 minutes and discard the flow through and reassemble the spin column with its
 collection tube.

Step 5: Washing

- Add 500 μl of Wash Buffer to the column, centrifuge at 8000 g for 1 minutes and discard the flow through
 and reassemble the spin column with its collection tube.
- Repeat step 5.

Step 6: Column Drying

- Centrifuge at **8000 g** for **1** minute.
- Discard the collection tube and transfer the spin column to a new 1.5 ml microcentrifuge tube.

Step 7: **DNA Elution**

- Add 50 µl of Elution Buffer to the center of column and let stay at RT for 3 minutes.
- Centrifuge at **10000 g** for **2** minutes.

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