Azma Elixir Pajooh

RNrich FFPE Kit

Cat. No. AEFRX-1156

For Research Use Only

Introduction

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

RNrich FFPE Tissue Kit components*
Cat. No. AEFRX-1156-50

G Solution	**50 ml**
ZR Solution(conc.) **	**15 ml**
TR Buffer	**20 ml**
Activator Reagent**	**2 ml**
VR 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.
** Please refer to reminder, **Activator Reagent, Wash Buffer and ZR Solution Preparation** before using this kit

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 RNA extraction, **never** open the microtube cap outside the laminar hood.
- To have a high and pure yield all centrifuge step should be done at **4°C**.

Activator Reagent Preparation

- Add **2** ml of **molecula**r 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:

	**Catalogue number**
	**Manufacturer**
	**Temperature limit**
	**Batch code**
	**Use-by date**
	**Contains sufficient for <n> tests**
	**Consult instructions for use**

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Protocol

Step a: **Sample Preparation**

1a. Transfer **50 mg** (up to 200 mg) of tissue sample into a 1.5 ml tube.

Step b: **Paraffin Removal**

b1 Add **500 µl G Solution** to sample tube and vortex vigorously.
b2 Incubate at **65°C** for **2** minutes**.**
b3 Centrifuge at **12000** g for **5** minutes.
b4 **Decant** the supernatant into waste.
b5 **Repeat step b**.

Step c: **Tissue Washing**

c1 Add **500 µl ZR Solution** to sample tube and vortex it.
c2 Centrifuge at **12000** g for **5** minutes.
c3 **Decant** the supernatant into waste.
Note: Repeat this step **to extract a pure RNA**.

Step d: **Tissue Digestion & Lysis**

Note: Refer to reminder before use activator reagent

d1 Add **400 µl** of **TR Buffer** and **40 µl** of **Activator Reagent** to the sample tube and vortex vigorously.
d2 Incubate at **65**°C for **30** minutes.
Note: During incubation time, vortex the sample tube every 10 minutes.
d3 Incubate at **85°C** for **10** minutes **and** vortex every 5 minutes**.**
d4 Add **200 µl** of **VR Buffer** to sample tube and vortex vigorously and then keep at RT for **5** minutes.
d5 Centrifuge at **4°C** and **12000 g** for **10** minutes.
Note: Do not disturb the phases.
d6 Carefully transfer about **300µl of supernatant** to a new 1.5 ml tube.
d7 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**.
d8 Centrifuge at **4°C** and **2000 g** for **2** minutes and **discard** the flow through and reassemble the spin column with its collection tube

Step e**: Washing**

e1 Add **500 µl** of **Wash Buffer** to the column, centrifuge at **4°C** and **8000 g** for **1** minutes and **discard** the flow through and reassemble the spin column with its collection tube.
e2 **Repeat step e1**.

Step f: **Column Drying**

f1 Centrifuge at **4°C** and **8000 g** for **1** minute.
f2 **Discard** the collection tube and transfer the spin column to a new 1.5 ml microcentrifuge tube.

Step g: **RNA Elution**

g1 Add **50 µl** of **Elution Buffer** to the center of column and let stay at **RT** for **3** minutes.
g2 Centrifuge at **4°C** and **10000 g** for **2** minutes.

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