Azma Elixir Pajooh

Cat. No. AFTRX-1152

For Research Use Only

Fast RNrich Tissue Kit

Introduction

The Fast **RNrich** Tissue Kit provides all of the reagents necessary to extract RNA from a wide variety of biological sources. RNA purified with this kit is suitable for a variety of applications, including amplification and digestion with restriction endonucleases.

Fast RNrich Tissue Kit components **Cat. No. AFTRX-1152-50**

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**
Manual	**1**
column	**50**

* Refer to reminder, **Activator Reagent Preparation** and **Wash Buffer Preparation** before first use.

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.

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 Preparation

Add **35 ml** molecular biology grade **Absolute Ethanol** to **Wash Buffer** bottle before first use and mark the check box on it.

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PROTOCOL

Step a: **Sample Preparation**

a1 Transfer **50 mg** (up to 200 mg) of tissue sample into to a 1.5 ml tube.

Step b: **Tissue Digestion & Lysis**

b1 Add **400 µl** of **TR Buffer** and **40 µl** of **Activator Reagent** to the sample tube and vortex vigorously.
b2 Incubate at **65**°C until tissue are completely lysed (usually 30 to 120 minutes).
Note: During incubation time, vortex the sample tube every 10 minutes.
b3 Incubate at **85°C** for **10** minutes **and** invert every 5 minutes**.**
b4 (**Optional**) Add **200 µl** of **VR Buffer** to sample tube and vortex vigorously and then keep at RT for **10** minutes.
b5 Centrifuge at **10000 g** for **10** minutes.
Note: Do not disturb the phases.
b6 Carefully transfer about **300µl of supernatant** to a new 1.5 ml tube.
b7 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**.
b8 Centrifuge at **2000 g** for **2** minutes and **discard** the flow through.

Step c**: Washing**

c1 Add **500 µl** of **Wash Buffer** to the column, centrifuge at **8000 g** for **2** minutes and **discard** the flow through.
c2 **Repeat step c1**.

Step d: **Column Drying**

d1 Centrifuge at **8000 g** for **1** minute.
d2 **Discard** the flow through and place the column into a new **1.5 ml** microcentrifuge tube.

Step e: **RNA Elution**

e1 Add **50 µl** of **Elution Buffer** to the center of column and let stay at **RT** for **3** minutes.
e2 Centrifuge at **10000 g** for **2** minutes.
Note: Eluted RNA is ready for downstream analysis and should be stored at **-20°C**.

Short PROTOCOL

Step a: **Sample Preparation**

a1 Transfer **50 mg** (up to 200 mg) of tissue sample into to a 1.5 ml tube.

Step b: **Tissue Digestion & Lysis**

b1 Add **400 µl** of **TR Buffer** and **40 µl** of **Activator Reagent** to the sample tube and vortex vigorously.
b2 Incubate at **65**°C until tissue are completely lysed (usually 30 to 120 minutes).
Note: During incubation time, vortex the sample tube every 10 minutes.
b3 Incubate at **85°C** for **10** minutes **and** invert every 5 minutes**.**
b4 Centrifuge at **10000 g** for **10** minutes.
Note: Do not disturb the phases.
b5 Carefully transfer about **300µl of supernatant** to a spin column.
b6 Centrifuge at **2000 g** for **2** minutes and **discard** the flow through.

Step c**: Washing**

c1 Add **500 µl** of **Wash Buffer** to the column, centrifuge at **8000 g** for **2** minutes and **discard** the flow through.
c2 **Repeat step c1**.

Step d: **Column Drying**

d1 Centrifuge at **8000 g** for **1** minute.
d2 **Discard** the flow through and place the column into a new **1.5 ml** microcentrifuge tube.

Step e: **RNA Elution**

e1 Add **50 µl** of **Elution Buffer** to the center of column and let stay at **RT** for **3** minutes.
e2 Centrifuge at **10000 g** for **2** minutes.
Note: Eluted RNA is ready for downstream analysis and should be stored at **-20°C**.

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