Cat. No. AETRX-1102

For Research Use Only

RNrich Tissue Kit

Azma Elixir Pajooh

Introduction

The **RNrich** Tissue Kit provides all of the reagents necessary to extract total RNA from a wide variety of animal tissues. RNA purified with this kit is suitable for a variety of applications, including amplification and total cDNA synthesis.

RNrich Tissue Kit components* **Cat. No. AEGTRX-1102-050**

TR Buffer	**20 ml**
Activator Reagent**	**2 ml**
VR Buffer	**10 ml**
PE Buffer	**20 ml**
Wash Buffer(conc.) **	**15 ml**
Elution Buffer	**3 ml**
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 Preparation** and **Wash Buffer 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.
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.
Note: For the best results, the prepared Activator Reagent should be used immediately. Prepared Activator Reagent can be stored for up to 6 months at -20°C.

Wash Buffer Preparation

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

PROTOCOL

Step 1: Sample Preparation

- Transfer **50 mg** (up to 200 mg) **of fresh tissue** to a 1.5 ml microcentrifuge tube.

Step 2: **Tissue Digestion**

Note: Refer to reminder before using activator reagent for the first time.

- Add **400 µl** of **TR Buffer** and **40 µl** of prepared **activator reagent** to the sample tube and vortex.
- Incubate at **65°C** until tissues are completely lysed (usually **30 to 90** minutes**).**
  Note: During incubation time, vortex vigorously the sample tube every 10 minutes.
- Incubate at **85°C** for **10** minutes**.**

Step 3: **Lysis**

- Add **200 µl** of **VR Buffer** to the sample tube and mix by vortex.
- Keep at **room temperature** for **10** minutes and **invert** each 5 minutes.
- Centrifuge at **11500 g and 4 °C** for **15** minutes.
- Carefully transfer **400 µl of supernatant** to a new **1.5** microcentrifuge tube and do not disturb the phases.

Step 4: **RNA Precipitation**

- Add **400 µl of PE Buffer to** the sample tube and **invert slowly** for 15 times.
- Incubate the samples at **-20°C** for **30** minutes.
  Note: In sample with low amount of RNA, incubation time can be up to overnight.
- Centrifuge at **12000 g and 4 °C** for **15** minutes.
- **Decant** the supernatant into waste.
  Note: While decanting, be careful not to lose the RNA pellet.

Step 5: **Wash**

Note: Refer to reminder before use **Wash Buffer** for the first time.

- Add **500 µl** of **Prepared Wash Buffer** to the sample tube, invert slowly for 10 times and then centrifuge at **12000 g and 4 °C** for **10 minutes**.
- **Decant** the supernatant into waste.
- **Repeat Step 5**.
- **Decant** the supernatant into waste, and let stand until dry.
  Note: Do not heat to dry**.**
  Note: Do not open the cap of tube outside of laminar hood.

Step 6: **RNA Elution**

- Add **50 µl of Elution Buffer** to the sample tube.
- Resuspend the RNA for **5** minutes at **room temperature**, and then centrifuge at **8000 g** for **1** minute.
  Note: Store RNA at **-20°C**