Cat. No. AETDX-1009

For Research Use Only

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DNrich General Kit

Introduction

The **DNrich** General Kit provides all of the reagents necessary to extract total DNA from a variety of biological samples. DNA purified with this kit is suitable for many applications, including amplification and digestion with restriction endonucleases.

DNrich General Kit components*	**Cat. No. AETDX-1009-050**
TD Buffer	**20 ml**
Activator Reagent**	**2 ml**
VI Buffer	**15 ml**
PE Buffer	**15 ml**
Wash Buffer (conc.) **	**15 ml**
Elution Buffer	**3 ml**
Manual	**1**

* 1.5 ml microcentrifuge tube and molecular biology grade water and **Absolute Ethanol** are needed but are not included.
** Please refer to **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.

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.

PROTOCOL

Step 1: **Sample Preparation**

- Transfer **50 mg** (up to 200 mg) or **100 µl** of homogenized sample into a sterile 1.5 ml tube.

Step 2: **Digestion**

- Add **400 µl** of **TD Buffer** and **40 µl** of **prepared activator reagent** to the sample tube and vortex vigorously.
- **Incubate** at **65°C** until tissue completely lysed (usually 20 to 120 minutes).
  Note: During incubation time, vortex the sample tube every 10 minutes.
- Incubate at **85°C** for **10** minutes to inactivate activator reagent.

Step 3: **Lysis**

- Add **300 µl** of **VI Buffer** to the sample tube and mix by vortex.
- Keep at **room temperature** for 10 minutes and **invert** each 5 minutes.
- Centrifuge at **11000 g** for **10** minutes.
- Carefully transfer about **300 µl** of supernatant to a new 1.5 microcentrifuge tube and do not disturb the phases.

Step 4: **DNA Precipitation**

- Add **300 µl of PE Buffer to** the sample tube and mix by **invert** for 15 times.
- Incubate the samples at **room temperature** for **30 to 60** minutes.
  Note: In sample with low amount of DNA, incubation time can be up to overnight at **-20°C**.
- Centrifuge at **12000 g** for **20** minutes.
- **Decant** the supernatant into waste.
  Note: While decanting, be careful not to lose the DNA pellet.

Step 5: **Wash**

Note: Prepare **Wash Buffer** before first use

- Add **500 µl** of **Wash Buffer** to the column, invert for 10 times and then centrifuge at **12000 g** for **10** minutes.
- **Decant** the supernatant into waste.
- **Repeat step 5**.

Step 6: **Drying**

- With the cap open, let stand until dry at room temperature or incubate at **55°C** for up to **10** minutes.

Step 7: **DNA Elution**

- Add **50 µl of Elution Buffer** to the sample tube.
- Resuspend the DNA for 5 minutes at 55°C, and then centrifuge at **8000 g** for 1 minute.
  Note: Store DNA at **-20°C**