





#### Introduction

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

**Fast DNrich General Kit components** 

Cat. No.	AF	GDX-1	054-050
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TD Buffer	20 ml	
Activator Reagent*	2 ml	
VI Buffer	10 ml	
SE Buffer	15 ml	
Wash Buffer (conc.) *	15 ml	
Elution Buffer	3 ml	
column	50	
Manual	1	

<sup>\*</sup> 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.

# **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 a: Sample Preparation

a1 Transfer **50 mg** (up to 200 mg) of tissue or **100 \mul of** homogenized sample into a 1.5 ml tube.

# Step b: Tissue Digestion & Lysis

- b1 Add 400 μl of TD 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 **VI Buffer** to sample tube and vortex vigorously and then keep at RT for **5** minutes.
- b5 Centrifuge at **11000 g** for **5** 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  $500 \mu l$  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: DNA 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.

### **Short PROTOCOL**

## Step a: Sample Preparation

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

#### Step b: Tissue Digestion & Lysis

- b1 Add **400 μl** of **TD 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).
- b3 Incubate at 85°C for 10 minutes.
- b4 Centrifuge at 11000 g for 5 minutes.
- 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  $\mu$ l 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: **DNA Elution**

- e1 Add 50  $\mu$ l 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: Store DNA at -20°C.

