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DNrich Blood Kit

Introduction

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

DNrich Tissue Kit components* Cat. No. AEBDX-1005-050

TD Buffer	20 ml
Activator Reagent**	2 ml
VI Buffer	10 ml
PE Buffer	15 ml
Wash Buffer (conc.) **	15 ml
Elution Buffer	3 ml
Manual	1

* 1.5 ml microcentrifuge tube, molecular biology grade water, and 70% ethanol are needed but are not included.

** Please refer to **Activator Reagent 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 heater 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 at 4°C.

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 **200 µl** of whole Blood sample to a 1.5 ml microcentrifuge tube.
Note: The blood sample should be homogenized completely before use.

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** for **30**.
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 **200 µl** of **VI Buffer** to the sample tube and mix by vortex.
- Keep at **room temperature** for 15 minutes and **invert** each 5 minutes.
- Centrifuge at **11500 g** for **15** minutes.
- Carefully transfer about **300µl 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 **-20°C** for **30 to 60** minutes.
Note: In sample with low amount of DNA, incubation time can be up to overnight.
- Centrifuge at **12000 g** for **15** minutes.
- **Decant** the supernatant into waste.
Note: While decanting, be careful not to lose the DNA pellet.

Step 5: Wash

- Add **500 µl of Wash Buffer** to the sample tube, invert for 10 times and then centrifuge at **12000 g** for 10 minutes.
- **Decant** the supernatant into waste.
Note: Repeat step **5** to have a high-purity DNA.
- After decantation let stand until dry.

Step 6: DNA Elution

- Add **50 µl of Elution Buffer** to the sample tube.
- Resuspend the DNA for 5 minutes at **55°C** or room temperature, and then centrifuge at **10000 g** for 1 minute.
Note: Store DNA at **-20°C**.

