Cat. No. AESDX1010

For Research Use Only

DNrich Saliva Kit

Azma Elixir Pajooh

Introduction

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

DNrich Saliva Kit components*	**Cat. No. AESDX1010-50**
ST Solution	50 ml
TD Buffer	20 ml
Activator Reagent**	2 ml
VI Buffer	15 ml
PE Buffer	25 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 **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 -20°C.

Activator Reagent Preparation

Add **1.5** 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 Add **2 ml** of saliva sample to a sterile 2 ml microcentrifuge tube then Centrifuge at **10000 g** for **5** minutes, **Decant** the supernatant into waste.
a2 Add **1000 µl ST Solution** to the sample tube and vortex it well.
a3 **Transfer 300 µl** of **sample** to a new 1.5 ml microcentrifuge tube.

Step b: **Digestion**

b1 Add **400 µl** of **TD Buffer** and **40 µl** of **prepared activator reagent** to the sample tube and vortex vigorously.
b2 **Incubate** at **65°C** for **30** minutes**.** During incubation time, vortex the sample tube every 10 minutes.
b3 **Incubate** at **85°C** for **10** minutes**.**

Step c: **Lysis**

c1 Add **300 µl** of **VI Buffer** to the sample tube and mix by vortex.
c2 Keep at **room temperature** for 10 minutes and **invert** regularly.
c3 Centrifuge at **11500 g** for **5** minutes.
c4 Carefully transfer about **500µl of supernatant** to a new 1.5 microcentrifuge tube and do not disturb the phases.

Step d: **DNA Precipitation**

d1 Add **500 µl of PE Buffer to** the sample tube and mix by **invert** for **15 times.**
d2 Keep at **room temperature** for **10** minutes.
Optional (for stale samples): Incubate the samples at **-20°C** for **30 to 60** minutes.
d3 Centrifuge at **12000 g** for **10** minutes.
d4 **Decant** the supernatant into waste.
Note: While decanting, be careful not to lose the DNA pellet.

Step e: **Wash**
Note: Prepare **Wash Buffer** before first use
e1 Add **500 µl** of **prepared Wash Buffer** to the sample tube, invert for 10 times and then centrifuge at **12000 g** for **5** minutes.
e2 **Decant** the supernatant into waste.
e3 **Repeat step e1.**
e4 **Decant** the supernatant into waste, and let stand until dry.

Step f: **DNA Elution**

f1 Add **50 µl of Elution Buffer** to the sample tube.
f2 Resuspend the DNA for **5 minutes** at **room temperature** or **at 55**°C, and then centrifuge at **8000 g** for **1 minute**.
Note: Store DNA at **-20°C.**