Cat. No. AESDX1012

For Research Use Only

DNrich Sperm Kit

Azma Elixir Pajooh

Introduction

The **DNrich** Sperm Kit provides all of the reagents necessary to extract DNA from Semen. The semen samples don't need treatment to be used with this kit. The DNA purified with this kit is suitable for a variety of applications, including amplification and digestion with restriction endonucleases.

DNrich Sperm Kit components*	**Cat. No. AESDX1012-50**
PB Buffer	7.5 ml
Activator Reagent**	1 ml
VR Buffer	10 ml
PE Buffer	10 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** ml of **molecular biology grade water** to **Activator Reagent**, and mix 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 Semen sample to a sterile 2 ml microcentrifuge tube then Centrifuge at **12000 g** for **10** minutes, **pipette of** the supernatant into waste, be careful not to lose the cell pellet.

Step b: **Digestion**

b1 Add **150 µl** of **PB Buffer** and **20 µl** of **prepared activator reagent** to the sample tube and vortex vigorously.
b2 **Incubate** at **65°C** for **overnight.**
b3 **Incubate** at **85°C** for **10** minutes**.**

Step c: **Lysis**

c1 Add **200 µl** of **VR 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 **15 minutes**.
c4 Carefully transfer about **200 µl of supernatant** to a new 1.5 ml microcentrifuge tube and **do not disturb the phases**.

Step d: **DNA Precipitation**

d1 Add **200 µl of PE Buffer to** the sample tube and mix by **invert**.
d2 Keep at **-20°C** for **60 minutes**.
d3 Centrifuge at **12000 g** for **15** 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 **10** 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 **30 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.**