Cat. No. AEDX-1006

For Research Use Only

Azma Elixir Pajooh

DNrich Gelatin Kit

Introduction

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

DNrich Gelatin Kit components **Cat. No. AEGEDX1006- 50**

TG Buffer	25 ml
Activator Reagent*	2 ml
LS Buffer	40 ml
VI Buffer	25 ml
SG Buffer (conc.) *	10 ml
Wash Buffer (conc.) *	15 ml
Elution Buffer	2 ml
Manual	1

* Please refer to **Activator Reagent, SG Buffer** 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.

Wash Buffer and SG Buffer Preparation

Add **35 ml** molecular biology grade **Absolute Ethanol** to each **Wash Buffer** and **SG Buffer** bottle **before first use** and mark the check box on it.

Activator Reagent Preparation

Before first use, add **2 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.

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PROTOCOL

Step 1: **Sample Preparation**

• Weight **0.07 mg** of gelatin powder CRM (and transfer it to a 2 ml microcentrifuge tube.

Step 2: **Lysis**

First Step

• Add **500 µl** of **TG Buffer** and **40 µl** of **prepared activator reagent** to the sample tube and vortex vigorously.

• Incubate at **65°C** for **45 to 60** minutes**.**

Note: During incubation time, vortex the sample tube every 10 minutes.

• Incubate at **85°C** for **10** minutes**.**

Second Step

• Add **800 µl** of **LS Buffer** to the sample tube and mix by vortex.

Note: **Shake** the **LS** bottle before use and keep it`s cap tight closed after use.

• Keep at **room temperature** for **10** minutes.

• Centrifuge at **11500 g** for **10** minutes.

Note: All centrifuge steps should be done at room temperature.

• Carefully transfer the **supernatant** to a new 1.5 microcentrifuge tube, **do not disturb the phases**.

Third Step (OPTIONAL)*

***This step is necessary to achieve higher concentration of nucleic acid**

• Add **500** µl of **VI Buffer** to the sample tube, invert for 10 minutes.

Note: keep the cap of VI buffer bottle tight closed after use.

• Centrifuge at **12000 g** for **10** minutes.

• Again, transfer up to **500** µl of the **supernatant** to a new 1.5 microcentrifuge tube, do not disturb the phases.

Step 3: **DNA Precipitation**

Note: SG Buffer should be prepared before first use.

Note: Keep **SG Buffer** at **room temperature** before use.

• Add **800 µl of SG Buffer to** the sample tube and mix by **invert** for 15 times.

• **Incubate** the samples at **room temperature** for **60** minutes.

• Centrifuge at **12000 g** for **25** minutes.

• **Decant** the supernatant into waste.

Note: While decanting, be careful not to lose the DNA pellet.

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Step 4: **Wash**ig

Note: **Wash Buffer** should be prepared before first use.

• Add **500 µl** of **Wash Buffer** to the sample tube, invert for 10 times and then centrifuge at **12000 g** for **15** minutes.

• **Decant** the supernatant into waste.

• Again 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, and let stand until dry.

Step 5: **DNA Elution**

• Add **30 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**.

Troubleshooting

Little or no DNA after resuspension in Elution buffer.

1) Increase the amount of sample up to 300 mg. Use the recommended amount of starting material or use the recommended ratio of LS buffer as indicated in the protocol.

NOTE: This protocol is optimized for DNA extraction from gelatin powder CRM, for other kind of samples the amount of sample and starting material should be optimized.

2) **Increase the efficiency of cell lysis.** Increase the time of incubation.

3) Ensure that DNA remains following SG buffer precipitation. Make certain that the nucleic acid pellet adheres to the microcentrifuge tube during washing step, period of centrifuge during precipitation and washing can be increased.