Gel Extraction Kit

Cat. No.: S-1050; 25 applications
Cat. No.: S-1050-1; 50 applications

DENA ZIST ASIA
MOLECULAR BIOLOGY PRODUCTS
www.denazist.ir

Manual

Description: DENAzist gel extraction kit is suitable for the recovery of nucleic acids from agarose gels. Nucleic acids are efficiently recovered from standard or low-melting gel agarose in either TAE or TBE buffer. Recovered nucleic acid can be used in a multitude of sensitive applications including: PCR and qPCR, transfection, plasmid subcloning, *in vitro* transcription, and Sanger sequencing.

VERSION: Nov 2021

Important instructions before first use

For 25 application kit, add 12 ml ethanol (96%-100%) to the container labeled GR2.
For 50 application kit, add 24 ml ethanol (96%-100%) to the container labeled GR2.
Label the container to record that ethanol has been added before first use.

PROTOCOL for recovery of nucleic acids from gel

Before the first use, make sure that ethanol has been added to the container labeled GR2.

1. Transfer a small volume of GR3 buffer into a microfuge tube and place the tube in a 60°C water bath. This will be used for elution.

------ DNA RELEASE FROM GEL ------
& BINDING TO THE COLUMN

2. Run nucleic acid samples on agarose gel. Weigh an Eppendorf tube and write down its weight. On a transilluminator using a clean scalpel blade cut the band containing the DNA fragment of interest, while avoiding to include extra pieces of agarose gel. Place the cut band into the Eppendorf tube. Weigh the tube. By subtracting the new and old weights, determine the weight of the agarose gel containing the nucleic acid. In this protocol, each 100 mg of agarose gel is considered to have a volume of 100 µl.

3. Add 1 gel volume from buffer **GR1** to the gel (for example, add 140 µl of buffer GR1 to 140 mg of gel). If the gel concentration is more than 2%, add 6 volumes from buffer GR1. Place the tube containing the gel and buffer GR1 for 20-30 min in a water bath set to 60 °C. Occasionally vortex the tube during the incubation time. Make sure that the gel slice dissolves completely. If not, increase the incubation time.

4. When the gel slice is completely dissolved, add 10 µl from **sodium acetate** solution and mix. Add equal to the gel volume from 96-100% ethanol. Mix by pipetting.

5. Mount a spin column onto a collection tube (both are included in the kit). Using a sampler (pipettor), transfer the whole mix to the upper reservoir of the spin column. Centrifuge the spin column at 8,000 rpm for 2 minutes. Discard the flow-through accumulated in the collection tube.

-------WASHING-------

6. Remount the spin column onto the collection tube. Add 700 µl from **GR2** buffer onto the spin column. Let the tube stand at room temperature for 2 minutes. Centrifuge the spin column and its collection tube at 10,000 rpm for 1 minute. Discard the flow-through.

7. Without adding any solution, centrifuge the tube one more time at top speed (13,000 -14,000 rpm) for 3 minutes.

-------ELUTION-------

8. Separate the spin column from its collecting tube and place it into a new 1.5 ml microfuge (Eppendorf) tube. Add 40-100 µl from pre-warmed (to 60 °C) **GR3** solution onto the center of spin column. Leave the spin column (mounted on the microfuge tube) for 5 minutes at 60 °C. Centrifuge the spin column at 10,000 rpm for 2 minutes.

Important: The volume for elution will determine the final concentrtaion. If higher concentration of DNA is needed, the volume for elution could be 40-50 µl.

9. Return the eluted solution back on to the center of spin column. Leave the spin column mounted on the microfuge tube for 4 min at room temperature. Centrifuge the spin column mounted on the microfuge tube at 13,000 rpm for 2 minutes.

10. Discard the spin column. The eluted solution at the bottom of the microfuge tube will contain the purified nucleic acid. Until further downstream processes store the sample in -20 °C freezer. ◢

Kit components:

Buffers: GR1, GR2,GR3
Na acetate
Spin Columns

Store all components at room temperature

Notes: Research Use Only
This product insert declares that this products has been analyzed and passed the quality control tests at the time of manufacture.