DENAzist Gel-Max-Extract Kit

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

Manual

Description: DENAzist Gel-Max-Extract Kit is highly efficient in recovering nucleic acids from standard or low-melting agarose gels in either TAE or TBE buffer. Recovered nucleic acids can be used in a multitude of sensitive applications, including PCR and qPCR, transfection, plasmid subcloning, in vitro transcription, and Sanger sequencing. DENAzist Gel-Max-Extract Kit replaces the previous DENAzist Gel Extraction Kit.

VERSION: Nov. 2024

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.

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PROTOCOL for recovery of nucleic acids from gel

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

DNA RELEASE FROM GEL & BINDING TO THE COLUMN

1. Run nucleic acid samples on an agarose gel. Weigh an Eppendorf tube and record its weight. Using a clean scalpel blade on a transilluminator, cut the band containing the DNA fragment of interest, avoiding extra pieces of agarose gel. Place the cut band into the Eppendorf tube. Weigh the tube again. Determine the weight of the agarose gel containing the nucleic acid by subtracting the old weight from the new weight. In this protocol, each 100 mg of agarose gel is considered to have a volume of 100 µl.
2. Add 3 volumes of buffer **GR1** to the gel (e.g., add 300 µl of buffer GR1 to 100 mg of gel). If the gel concentration is more than 2%, add 6 volumes of buffer GR1. Place the tube containing the gel and buffer GR1 in a water bath set to 50 °C for 10 minutes, occasionally vortexing the tube during the incubation. Ensure the gel slice dissolves completely. If not, increase the incubation time.
3. Once the gel slice has fully dissolved, add a volume of **sodium acetate** solution equal to 1/20th of the combined gel and GR1 volumes, then mix well. For example, to 100 mg of gel plus 300 µl of buffer GR1, add 20 µl of sodium acetate solution.
4. Add a volume of **ethandol** equal to 1/2 of the combined gel and GR1 volumes, then mix well. For example, to 100 mg of gel plus 300 µl of buffer GR1, add 200 µl of ethanol.
5. Mount a spin column onto a collection tube (both included in the kit). Using a pipettor, transfer the entire mix to the upper reservoir of the spin column. Centrifuge the spin column at 8,000 rpm for 1 minute. Discard the flow-through accumulated in the collection tube

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

6. Remount the spin column onto the collection tube. Add 700 µl of **GR2** buffer to the spin column. Centrifuge the spin column and collection tube at 8,000 rpm for 1 minute. Discard the flow-through
7. Without adding any solution, centrifuge the tube once more at top speed (13,000-14,000 rpm) for 3 minutes.

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

8. Separate the spin column from its collection tube and place it into a new 1.5 ml microfuge (Eppendorf) tube. Add 50 µl of **GR3** solution to the center of the spin column. Leave the spin column (mounted on the microfuge tube) for 5 minutes. Centrifuge the spin column at 13,000 rpm for 2 minutes.
9. Discard the spin column. The eluted solution at the bottom of the microfuge tube will contain the purified nucleic acid. Store the sample in a -20 °C freezer until further downstream processes. ◢

Kit components	25 app.	50 app.
GR1 buffer	12.5 ml	25 ml
GR2 buffer	9 ml	18 ml
GR3 buffer	3 ml	6 ml
Na Acetate solution	0.7 ml	1.4 ml
Spin column	25	50

Store all components at room temperature.
Expiry date: 18 month from the date of manufacture

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.