Bacterial DNA Isolation Kit

Cat. No.: S-1035-5; 5 applications
Cat. No.: S-1035; 25 applications
Cat. No.: S-1035-1; 50 applications

DENA ZIST ASIA
MOLECULAR BIOLOGY PRODUCTS
www.denazist.ir

Expiry date: 18 month after shipping

Manual

Description: DENAzist Bacterial DNA Isolation Kit is designed for the extraction of genomic DNA from microorganisms and gram positive and negative bacteria in biological fluids including ruminal fluid, urine, saliva, and sputum and cultured colonies, and microorganisms with hard cell wall including mycobacteria in biological fluids or cultured colonies. The extracted DNA could be used in a multitude of downstream procedures including PCR, genotyping, DNA digestion, and sequencing.

VERSION: Sept 2023

Important instructions before first use

For 5 application kit, add 3.6 ml ethanol (96%-100%) to DB3 container. For 25 application kit, add 18 ml ethanol (96%-100%) to DB3 container. For 50 application kit, add 36 ml ethanol to DB3 container.
Label the container to record that ethanol has been added before first use.

PROTOCOL for isolation of Genomic DNA from bacteria

Before the first use, make sure that ethanol has been added to the container labeled DB3.

-------PREPARATION & DNA RELEASE -------

1. Add 0.7 ml of the **DB1 buffer** to a microcentrifuge tube. Add 7 µl from 2-mercaptoethanol (not provided in the kit) and mix.
2. Transfer 2 mL of biological fluids or 1 ml of bacterial liquid culture to a 2-ml microcentrifuge tube. Centrifuge the tube at 13,000 RPM for 2 minutes. Discard the supernatant and keep the pellet.
3. Resuspend the pellet of bacterial cells in 0.7 ml of the mix from step 1. Add glass beads and vortex at maximum speed (5 minutes for Gram negative, and 10 minutes for Gram positive bacteria).
4. Add 10 µl of **RNaseA** to the lysate, mix by pipetting, and incubate for 15-20 minutes at 37 °C.
5. Add 10 µl **proteinase K**, mix by pipetting, and incubate the tube at 60 °C (20 minutes for Gram negative, and 60 minutes for Gram positive bacteria).
6. Centrifugate the tube for 5 min at 13,000 rpm. Transfer the supernatant to a new 2-ml tube. Discard the pellet.
7. Add 0.7 ml from the **DB2 buffer.** Mix the contents by pipetting. Add 0.7 ml from ethanol. Mix by pipetting.

--DNA BINDING TO THE COLUMN--

8. Transfer 700 µl of the lysate into a spin column (inserted into a collection tube, both provided in the kit). Centrifuge the spin column at 10,000 rpm for 1 minute. Discard the flow-through accumulated in the collection tube. Remount the spin column onto the collection tube.
9. Transfer the rest of the lysate by repeating step 8.

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

10. Add 700 µl from the **DB3 solution** into the spin column. Centrifuge the spin column at 10,000 rpm for 1 minute. Discard the flow-through.
11. Add 500 µl from the **DB3 solution** into the spin column. Centrifuge the spin column at 10,000 rpm for 1 minute. Discard the flow-through.
12. Without adding any solutions, spin the tube one more time at top speed (13,000 rpm) for 5 minutes.

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

13. Separate the spin column from its collecting tube and place it into a new 1.5 ml microfuge (Eppendorf) tube.
14. Add 50-100 ul from the **DB4 solution** onto the center of the spin column. Leave the spin column mounted on the microfuge tube for 5 min at room temperature. Centrifuge the spin column mounted on the microfuge tube at 10,000 rpm for 2 min.
15. Return the eluted solution from the previous step back onto the center of the spin column. Leave the spin column mounted on the microfuge tube for 3 min at room temperature. Centrifuge the spin column mounted on the microfuge tube at 13,000 rpm for 2 min.
16. The eluted solution at the bottom of the microfuge tube contains genomic DNA. Until further downstream processes store the sample at -20 °C freezer.

Kit components:

	5 app.	25 app.	50 app.
DB1	4 ml	20 ml	40 ml
DB2	3.6 ml	18 ml	36 ml
DB3	2.7 ml	13.5 ml	27 ml
DB4	1 ml	3 ml	6 ml
Proteinase K	55 µl	275 µl	550 µl
RNase A	55 µl	275 µl	550 µl
Glass beads	√	√	√
Spin columns	5	25	50

Store all components at room temperature except the enzymes which should be stored at -20 °C.

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.