Blood DNA Isolation Kit

Cat. No.: S-1031-5; 5 applications
Cat. No.: S-1031; 25 applications
Cat. No.: S-1031-1; 50 applications
Cat. No.: S-1031-250; 250 applications

DENA ZIST ASIA
MOLECULAR BIOLOGY PRODUCTS
www.denazist.ir

Manual

Description: DENAzist Blood DNA Isolation Kit is designed for the extraction of genomic DNA from whole blood (citrate, heparin, or EDTA added) or cultured mammalian cells. Using this kit, the highest amount (yield) of genomic DNA with the highest quality is extracted in the least amount of time. 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 the 5 application kit, add 2.4 ml ethanol (96%-100%) to the container labeled BD3. For the 25 application kit, add 12 ml ethanol (96%-100%) to the container labeled BD3. For the 50 application kit, add 24 ml ethanol (96%-100%) to the container labeled BD3. For the 250 application kit, add 60 ml ethanol (96%-100%) to each BD3 container. Label the container to record that ethanol has been added before first use.

PROTOCOL for isolation of blood DNA

Before first use, make sure that ethanol has been added to the container labeled BD3.

1. Add 5 µl **proteinase K** to a 1.5 ml microfuge tube.
2. Perform "a" or "b".
   a-Blood sample: Add 300 µl of fresh (or properly stored) whole blood. Mix for 5 seconds.
   b-Cultured cells: Add pellet of cultured cells which is resuspended in 300 µl PBS. Mix for 5 seconds.

Important: Blood samples kept at the refrigerator for more than 2 days do not yield enough DNA. For longer than overnight storage, the blood samples should be kept at -20°C or preferably at -80°C.

3. Add 300 µl **BD1**. Mix for 5 seconds.
4. Add 600 µl **BD2** to the tube. Mix for 5 seconds.
5. Incubate the tube at 60 °C for 15-30 minutes.
6. Add 300 µl ethanol. Mix for 5 seconds.

-------DNA BINDING TO THE COLUMN------

7. Transfer 750 µl from the lysate into a spin column (inserted into a collection tube, both provided in the kit).
8. Centrifuge the spin column at 8,000 rpm for 2 min. Discard the flow-through accumulated in the collection tube. Re-mount the spin column onto the collection tube.
9. Transfer the rest of the lysate (750 µl) into the same spin column. Centrifuge the spin column at 8,000 rpm for 2 min. Discard the flow-through accumulated in the collection tube. Remount the spin column onto the collection tube.

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

10. Add 700 µl from **BD3** solution into the spin column. Centrifuge the spin column at 10,000 rpm for 1 min. Discard the flow-through.
11. Without adding any solutions, centrifuge the tube one more time at top speed (13,000 rpm) for 3 min.

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

12. Separate the spin column from its collecting tube and place it into a new 1.5 ml microfuge (Eppendorf) tube. Add 50-100 µl from **BD4** solution onto the center of the spin column. Leave the spin column mounted on the microfuge tube for 5 min at room temperature.
13. Centrifuge the spin column mounted on the microfuge tube at 13,000 rpm for 3 min.
14. The eluted solution at the bottom of the microfuge tube contains pure genomic DNA. Until further downstream processes store the sample in a -20 °C freezer.

Note: Very low levels of RNA contamination, if present, can be removed by adding RNase A (2 mg/ml, 2 µl, not provided in the kit) to the solution at the end of stage 5 and incubation at 37 °C for 15 minutes before moving to stage 6.

Kit components:

	5 app.	25 app.	50 app.	250 app.
BD1	1.8 ml	9 ml	18 ml	90 ml
BD2	3.6 ml	18 ml	36 ml	180 ml
BD3	1.8 ml	9 ml	18 ml	90 ml
BD4	1 ml	3 ml	6 ml	30 ml
Proteinase K	27.5 µl	138 µl	275 µl	1375 µl
Spin columns	5	25	50	250

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

Expiry date: 18 month after shipping

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.