Animal Tissue DNA Isolation Kit

Cat. No.: S-1033-5; 5 applications
Cat. No.: S-1033; 25 applications
Cat. No.: S-1033-1; 50 applications

DENA ZIST ASIA
MOLECULAR BIOLOGY PRODUCTS
www.denazist.ir

Expiry date: 18 month after shipping

Manual

Description: DENAzist Animal Tissue DNA Isolation Kit is designed for the extraction of genomic DNA from animal tissues and a wide variety of animal cell types. Using this kit it is possible to extract genomic DNA with the highest quality. The extracted DNA could be used in a multitude of downstream procedures including PCR, genotyping, sequencing, and different hybridization techniques including Southern blotting.

VERSION: Sept 2023

Important instructions before first use

For the 5 application kit, add 2.4 ml ethanol (96%-100%) to the container labeled AT3. For the 25 application kit, add 12 ml ethanol (96%-100%) to the container labeled AT3. For the 50 application kit, add 24 ml ethanol (96%-100%) to the container labeled AT3. Label the container to record that ethanol has been added before first use.

PROTOCOL for Isolation of Genomic DNA from Animal Cells/Tissues

Before first use, make sure that ethanol has been added to the container labeled AT3. Buffer AT1 and AT2 may form precipitates upon storage. If it is necessary, warm these buffers in a 60 °C water-bath until the precipitates are fully dissolved.

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

1. Weigh 10–15 mg tissue (~ 2 mm^3) in a 2 ml microcentrifuge tube and add 450 µl **AT1** to the tube.
2. Add **glass beads** and vortex for 3-5 minutes. Previous freezing of the tissue in liquid nitrogen would greatly enhance tissue disruption. For cultured cells, just collect them as a pellet and add 450 µl AT1.
3. Add 10 µl **proteinase K**, vortex for 10 seconds, and incubate the tube at 60 °C for 20 minutes. For some tissues, longer incubation times are needed to be dissolved.
4. When the incubation period is finished, add 450 µl **AT2** buffer. Mix the contents by pipetting. Incubate the tube at 60 °C for 5 minutes.
5. Centrifuge the tube for 2 minutes at 13,000 rpm. This step is critical to remove tissue debris. Transfer the supernatant into a new tube.
6. Add 10 µl **RNase A**. Mix for 5 seconds. Incubate the tube at 37 °C for 30 minutes.
7. Add 450 µl ethanol. Mix the contents by pipetting.
8. Transfer 700 µl from the lysate into a spin column (inserted into a collection tube, both provided in the kit). Centrifugate the spin column at 10,000 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 by repeating step 8.

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

10. Add 500 µl from the **AT3** solution into the spin column. Centrifuge the spin column at 10,000 rpm for 1 min. Discard the flow-through.
11. Add 200 ul from the AT3 solution into the spin column. Centrifuge the spin column at 10,000 rpm for 1 min. Discard the flow-through.
12. Without adding any solutions, spin the tube one more time at top speed (13,000 rpm) for 5 min.
13. Separate the spin column from its collecting tube and place it into a new 1.5 ml microfuge (Eppendorf) tube.

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

14. Add 50-100 µl from the **AT4** 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 1 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 3 min.
16. The eluted solution at the bottom of the microfuge tube contains genomic DNA. Until further downstream processes store the sample in -20 °C freezer. ◢

Kit components:

	5 app.	25 app.	50 app.
AT1	3 ml	15 ml	30 ml
AT2	3 ml	15 ml	30 ml
AT3	1.8 ml	9 ml	18 ml
AT4	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.