Animal Tissue RNA Isolation Kit

Cat. No.: S-1023-5; 5 applications
Cat. No.: S-1023; 25 applications
Cat. No.: S-1023-1; 50 applications

DENA ZIST ASIA
MOLECULAR BIOLOGY PRODUCTS
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Manual

Description: DENAzist's Animal Tissue RNA Isolation Kit contains spin columns and solutions for quick and easy isolation of total RNA from animal cells and tissues. The unique composition of buffers selectively isolates RNA from biological samples and leaves the final elute with the least amount of DNA and protein. The isolated total RNA will have the highest quality for any downstream experiment. The extracted RNA using this kit has been tested and qualified for downstream processes including RT-PCR and RT-qPCR. This kit is suitable for isolation of RNAs with low level of expression including lincRNAs and antisense lncRNAs.

VERSION: Sept 2023

Important instructions before first use

For the 5 application kit, add 2.4 ml ethanol (96%-100%) to the container labeled **AR3**. For the 25 application kit, add 12 ml ethanol (96%-100%) to the container labeled **AR3**. For the 50 application kit, add 24 ml ethanol (96%-100%) to the container labeled **AR3**. Label the container to record that ethanol has been added before first use.

PROTOCOL for Isolation of RNA from Animal Cells and Tissues

Before first use, make sure that ethanol has been added to the container labeled AR3. Warm AR2 buffer to 70 °C.

Note: For extraction from animal cells/tissue follow steps a-b and 1-8. For extraction from animal tissues follow steps I-III and 1-8. All steps should be performed in RNase-free conditions.

PREPARATION OF CULTURED ANIMAL CELLS

a. To a homogenizing vessel add 5-10 x 10^6 cells or a pellet of cells with approximate volume of 30 µl. Add 175 µl of **AR1** buffer to this vessel. Add 2 µl 2-mercaptoethanol (not provided in the kit) and mix.

b. Homogenize cells for 15 to 30 seconds. Incubate the tube at room temperature for 5 minutes. Transfer the homogenate into a new microfuge (1.5 ml) tube.

Follow protocol from "RNA BINDING TO COLUMN".

PREPARATION OF ANIMAL TISSUE

I. For solid tissues, grind 100 mg of tissue sample in a mortar and pestle in liquid nitrogen. If the tissue is soft, there is no need for grinding and the protocol can be started from step II.

II. Add 30 mg of ground solid tissue or soft tissue to a homogenizing vessel.

III. Add 175 µl of **AR1** buffer to this vessel. Add 2µl 2-mercaptoethanol (not provided in the kit) and mix. Homogenize the tissue for 15 to 30 seconds. Repeat the homogenization step. Incubate at room temperature for 5 minutes. Transfer the supernatant into a new microfuge (1.5 ml) tube.

Follow protocol from "RNA BINDING TO COLUMN".

-------RNA BINDING TO COLUMN-------

1. Add 350 µl of pre-warmed (to 70°C) **AR2** to the tube. Invert the tube 2-3 times.
2. Centrifuge the tube at 13,000 rpm for 5 minutes at room temperature. Transfer the supernatant (~ 500 µl) to a new microfuge (Eppendorf) tube. Discard the pellet which contains tissue debris. Add 235 µl from 95-100% ethanol. Invert the tube 2-3 times.
3. Transfer the whole volume onto a spin column inserted into a collecting tube (provided by the kit). Centrifuge the tube at 10,000 rpm for 2 minutes at room temperature. Discard the flow-through.

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

4. Add 550 µl **AR3** to the spin column. Centrifuge at 10,000 rpm for 2 minutes at room temperature. Discard the flow-through.
5. Add 200 µl **AR3** to the spin column. Centrifuge at 13,000 rpm for 3 minutes at room temperature. Discard the flow-through.

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

6. Transfer the column onto a new 1.5 ml microfuge tube. Add 50-100 µl **AR4** to the spin column. Incubate at room temperature for 2 minutes. Spin the tube at 10,000 rpm for 2 minutes at room temperature.
7. Return the eluted solution from the previous step onto the center of the spin column. Leave the spin column mounted on the microfuge tube for 2 min at room temperature. Centrifuge the spin column mounted on microfuge tube at 13,000 rpm for 3 min. The eluted sample will contain total RNA.
8. Determine RNA concentration and quality by spectrophotometry and gel electrophoresis. Keep aliquots of the extracted RNA in -80 °C. ◢

Kit components:

	5 app.	25 app.	50 app.
AR1	1 ml	5 ml	10 ml
AR2	1.8 ml	9 ml	18 ml
AR3	1.6 ml	8 ml	16 ml
AR4	1 ml	3 ml	6 ml
Spin columns	5	25	50

Store AR1 and AR4 at 4 °C, and AR2 and AR3 buffers at room temperature.

Expiry date: 24 month after shipping