Column RNA Isolation Kit

Cat. No.: S-1020-5; 5 applications
Cat. No.: S-1020; 25 applications
Cat. No.: S-1020-1; 50 applications
Cat. No.: S-1020-250; 250 applications

DENA ZIST ASIA
MOLECULAR BIOLOGY PRODUCTS
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Manual

Description: DENAzist Column RNA Isolation Kit contains spin columns and solutions for isolation of total RNA from a wide range of cells and tissues including animal, plant, bacterial and yeast cells. The specific composition of buffers selectively isolates RNA from biological samples. The isolated total RNA will have the highest quality for any downstream experiment. This kit has been tested and qualified for downstream processes including RT-PCR, RT-qPCR, next-generation sequencing, and isolation of RNAs with low level of expression including lincRNAs and antisense lncRNAs.

VERSION: Oct. 2022

Important instructions before first use

For the 5 application kit, add 2.4 ml ethanol (96%-100%) to the container labeled **DR2**. For the 25 application kit, add 12 ml ethanol (96%-100%) to the container labeled **DR2**. For the 50 application kit, add 24 ml ethanol (96%-100%) to the container labeled **DR2**. For the 250 application kit, add 60 ml ethanol (96%-100%) to **each DR2 container.** Label the container(s) to record that ethanol has been added before first use.

PROTOCOL for isolation of RNA

Note: All steps should be performed in RNase-free conditions. For extraction of high-quality RNA, it should be noted that specific requirements for extraction from plant tissues with high phenolic compounds and polysaccharides and also from very hard animal tissues should be adapted to the following basic protocol.

1. Initial preparation: perform one of the options a, b, or c.

   a . Cultured animal cells: After trypsinization and washing cells with PBS, add 5-10 million cells to a homogenizing vessel or a 1.5 ml tube. Add 1 ml of **DR1** buffer to this vessel.

   For cultured animal cells grown in a monolayer, it would be possible to add DR1 buffer (1 ml per 10 cm^2 of cultured cells) directly into the culture flask, once the medium has been removed. **DR1** buffer detaches the cells from the culture flask. However, for some cells using a cell scraper may be necessary. There is no need to wash the cells prior to the addition of DR1 buffer.

   b . Animal and plant tissues or bacterial and yeast cells: Grind 100 mg of tissue or pellet of bacterial or yeast cells in liquid nitrogen using a mortar and pestle. Add 50 mg ground tissue/cells or soft tissues to a homogenizing vessel or a 1.5 ml tube containing 1 ml of DR1 buffer.

   c . Soft and delicate tissues: If the tissue is soft, add 50 mg tissue to a homogenizing vessel or a 1.5 ml tube containing 1 ml of DR1 buffer. There is no need for grinding.

2. Homogenize cells for 15 seconds. Repeat the homogenization step. If homogenizer is not available, the cells in DR1 could be vortexed for 2x 1 min periods. Incubate the tube at room temperature for 5 minutes.
3. Transfer the homogenate into a new microcentrifuge (1.5 ml) tube. Centrifuge the tube at 12,000 rpm for 10 minutes at 4 °C. Transfer the supernatant into a new microcentrifuge (1.5 ml) tube. Discard the pellet containing tissue debris.
4. Add 200 ul chloroform (not provided in the kit) to the supernatant. Shake the tube vigorously for 15 seconds. Incubate the tube at room temperature for 3 minutes. Centrifuge the tube at 12,000 rpm for 15 minutes at 4 °C.
5. After centrifugation, three phases will form. While avoiding the interphase transfer the top phase into a new microcentrifuge tube.

Note: During the transfer it is very critical to avoid interphase contamination. To this end it is very important to use "10-100 µl pipette tips" instead of "100-1000 µl pipette tips". The latter kind of tips due to their height, volume, and narrow orifice produce a very high vacuum force, which pulls (even invisibly) the interphase into the top phase and causes contamination. Thus, it would be better to cut out the tip of the 10-100 µl tips with a scissor (~ 7 mm) and use these tips for transfer. Transfer the top aqueous phase to the new tube in 50 µl aspirations.

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

6. To the tube containing the top phase, add half the volume of the aqueous phase from 100% ethanol. Mix well by inverting the tube several times. Transfer the solution onto a spin column inserted into a collecting tube (provided by the kit). Centrifuge the tube at 8,000 rpm for 2 minutes at room temperature. Discard the flowtrough.

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

7. Add 500 µl **DR2** to the spin column. Centrifuge at 10,000 rpm for 1 minute at 4 °C. Discard the flow-through.
8. After adding 200 µl **DR2**, centrifuge the tube at 13,000 rpm for 3 minutes at 4 °C. Transfer the column into a new 1.5 ml microcentrifuge tube.

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

9. Add 50-100 µl **DR3** to the center of the column. Incubate at room temperature for 3 minutes. Centrifuge the tube at 13,000 rpm for 3 minutes at room temperature.
10. The eluted sample will contain total RNA. Store the extracted RNA in -80 °C freezer. ◢

Kit components:

	5 app.	25 app.	50 app.	250 app.
DR1	5.2 ml	26 ml	52 ml	255 ml
DR2	1.8 ml	9 ml	18 ml	90 ml
DR3	1 ml	3 ml	6 ml	30 ml
Spin columns	5	25	50	250

Store DR1 at 4 °C, and DR2 and DR3 buffers at room temperature.

Expiry date: 24 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.