DENAzist RNA-prime Kit

Cat. No.: S-1010-25; 25 applications
Cat. No.: S-1010-50; 50 applications

Manual

Description: The DENAzist prime-RNA kit is composed of two unique solutions for the isolation of RNA from a wide range of cells, animal and plant tissues, and microorganisms. The extraction is based on the improved and optimized use of chaotropic agents and aromatic organic compounds by precipitation. Using this kit, high-quality RNA species can be isolated, which are immediately usable in a variety of downstream processes. During the isolation process, DNA is actively separated from RNA, resulting in RNA of high quality and purity. DENAzist RNA-prime Kit replaces the previous DENAzist Total RNA Isolation Kit.

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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.

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PROTOCOL for isolation of total RNA

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 G1 buffer to this vessel.

     For cultured animal cells grown in a monolayer, the G1 buffer (1 ml per 10 cm^2 of cultured cells) can be directly added to the culture flask, once the medium has been removed. **G1** 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 before the addition of G1 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 **G1** 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 **G1** buffer. There is no need for grinding.
2. Homogenize cells for 1 min. Repeat the homogenization step. If homogenizer is not available, the cells in G1 could be vortexed for 2x 1 min periods. Incubate the tube at room temperature for 5 minutes. Transfer the homogenate into a new microcentrifuge (1.5 ml) tube.
3. Centrifuge the tube at 12,000 rpm for 5 minutes at 4 °C. Transfer the supernatant into a new microcentrifuge (1.5 ml) tube. Discard the pellet containing the tissue debris.
4. Add 200 µl 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 100-1000 µl pipette 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 sterile scissor (~ 7 mm) and use these tips for transfer. Transfer the top aqueous phase to the new tube in 50 µl aspirations.***
6. To the tube containing the top phase, add half the volume of the aqueous phase from isopropanol and the same from **G2** buffer (e.g. add 100 µl isopropanol and 100 µl G2 to 200 µl aqueous phase). Mix the contents and incubate the tube at room temperature for 10 minutes.
7. Centrifuge the tube at 12,000 rpm for 10 minutes at 4 °C. A glassy or white flaky pellet will form. Decant the supernatant.
8. Add 1 ml of 70% ethanol (made with ribonuclease-free water) to the pellet. Resuspend the pellet by tapping the tube. Centrifuge the tube at 12,000 rpm for 5 minutes at 4 °C. Decant the supernatant.
9. Repeat step 8. Try to remove as much ethanol as possible without losing the pellet (using 1-10 µl tips).
10. Dry the pellet by leaving the tube open at room temperature for 10-15 minutes. Dissolve the pellet in 30-100 ul of G3. The amount of added water will determine the final concentration of RNA. Store the extracted RNA in -80 °C freezer. ◢

Kit components:

	25 app.	50 app.
G1 buffer	26 ml	52 ml
G2 buffer	9 ml	18 ml
G3 (DEPC-water)	6 ml	12 ml

Store the G1 and G2 buffers at 4 °C , and G3 at -20 °C.
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.