TOOLSmart RNA Extractor

Cat. no. DPT-BD24
Storage: 4℃ for 18 months
Product Size: 2x100 ml

Introduction

The TOOLSmart RNA extractor was developed based on a regular nucleic acid extraction reagent, with the addition of an indicator. It has more favorable lysis ability and higher sensitivity and can be used to isolate total RNA from samples such as viruses, bacteria, plant tissue, and body fluids. During sample homogenization, the extractor maintains the integrity of RNA while disrupting cells and dissolving cell components. The TOOLSmart RNA extractor can be used to isolate RNA in both small (50–100 mg tissue or 5×10^6 cells) and large (≥1 g tissue or $\geq 10^7$ cells) samples of human, animal, plant, or bacteria species within 1 h. DNA and protein contamination is eliminated from RNA products. The isolated RNA can be used for Northern Blot, Dot Blot, poly(A) selection, in vitro translation, RNase protection assay, and molecular cloning.

Important Notes

1. Before the addition of chloroform, homogenized samples in the TOOLSmart RNA extractor can be stored at −70°C for at least 1 month.
2. The RNA can be stored in 75% ethanol for at least 1 year at −20°C, or for at least 1 week at 2–8°C.
3. Notes on the prevention of RNase contamination
    a. Change gloves regularly to avoid RNase contamination.
    b. Use RNase-free plastic and follow tips to avoid cross-contamination.
    c. RNA can be protected in the TOOLSmart RNA extractor, but the RNA must be stored or processed in RNase-free plastic or glassware. To wipe off the RNase, the glassware can be heated at 150°C for 4 h. The plastic can be dipped in 0.5 M NaOH for 10 min, washed thoroughly with RNase-free ddH_2O, and sterilized.
    d. Use RNase-free ddH_2O to confect the solution. Add DEPC to water in a clean glass container to a final concentration of 0.1% (v/v). Incubate overnight and autoclave for 15 min to remove any trace of DEPC.

Reagents to be Supplied by the User

1. Chloroform
2. Isopropyl alcohol
3. RNase-free ddH_2O
4. 75% ethanol (in RNase-free ddH_2O)

Protocol

1. Homogenizing the samples.
    a. Plants (taking leaves as an example): Place fresh leaves in liquid nitrogen and either grind thoroughly with a mortar and pestle or grind in the TOOLSmart RNA extractor after cutting the leaves into pieces. This process is recommended to be completed within 1 min. Use 1 mL of TOOLSmart RNA extractor per 100 mg of leaves.
    b. Tissues (taking rat liver as an example): Add 1 mL of TOOLSmart RNA extractor per 30–50 mg of liver sample. Homogenize the sample using a power homogenizer. In most cases, the volume of the tissue sample should not exceed 10% of the volume of the TOOLSmart RNA extractor.
    c. Adherent Cells (do not use more than 1×10^7 cells): Cells grown in a monolayer in cell culture vessels can be either lysed directly in the vessel (up to 10 cm in diameter) or trypsinized and collected as a cell pellet prior to lysis. Cells grown in a monolayer in cell culture flasks should always be trypsinized. The following methods can be used.

Method A: To lyse cells directly, Add 1 mL of TOOLSmart RNA extractor directly to the cells in the culture dish per 10 cm2 of culture dish surface area. Pipette the lysate up and down several times.

Note: The volume of the TOOLSmart RNA extractor should be determined according to the surface area rather than the number of cells. An insufficient volume can result in DNA contamination of the isolated RNA

Method B: To trypsinize and collect cells, determine the number of cells, aspirate the medium, and wash the cells with PBS. Aspirate the PBS and add 0.10%–0.25% trypsin in PBS. After the cells detach from the dish or flask, add the medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and centrifuge at 300 g for 5 min to remove the supernatant.

Note: Ensure the supernatant has been completely removed. Residual medium can lead to the incomplete lysis of cells and reduced RNA yield remains in the upper aqueous phase after centrifugation. However, when dealing with fat tissue, the upper phase is a lipid layer that should be discarded. Retain the clean homogenizing portion for the next step.

    d. Suspension cells: Harvest cells by centrifugation and remove the culture medium. Add 1 mL of

TOOLSmart RNA extractor per 1×10^7 cells of bacteria or 5×10^6 to 5×10^7 cells of animal, plant, or yeast samples. Do not wash cells before adding the TOOLSmart RNA Extractor to minimize the likelihood of mRNA degradation. Samples from some yeast and bacteria may need to be homogenized using a power homogenizer.

e. Blood: Take fresh blood and add three volumes of the TOOLSmart RNA extractor. Mix thoroughly. (Recommended amount: 0.6 mL of TOOLSmart RNA extractor to 0.2 mL of whole blood)

2. Incubate homogenized samples at 15°C–30°C for 5 min for the complete dissociation of the nucleoprotein complex.
3. Optional step: Centrifuge the sample at 12,000 rpm (approximately 13,400 g) for 10 min at 4°C. Transfer the supernatant to a fresh microcentrifuge tube.

Note: When preparing samples with high fat, protein, polysaccharide, or extracellular material contcentrations (e.g., muscle, fat tissue, or tuberous plant material), an additional round of centrifugation may be required to remove insoluble material from the samples. RNA typically remains in the upper aqueous phase after centrifugation. However, when dealing with fat tissue, the upper phase is a lipid layer that should be discarded. Retain the clean homogenizing portion for the next step.

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4. Add 0.2 mL of chloroform per 1 mL of TOOLSmart RNA extractor. Cap the tube carefully and vortex for 15 s. Incubate for 3 min at room temperature. If a vortex cannot be executed, shake the tube vigorously by hand for 2 min.

Note: To recover a higher RNA concentration, refer the TOOLS Locking PLGEL protocol and proceed to “step 6.”

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5. Centrifuge the sample at 12,000 rpm (approximately 13,400 g) for 15 min at 4°C. The mixture will separate into a lower pink phenol–chloroform phase, an intermediate phase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. Transfer the aqueous phase to a new tube.
6. Add an equal volume of 100% isopropanol to the aqueous phase. Mix thoroughly and incubate at room temperature for 10 min.
7. Centrifuge at 12,000 rpm (approximately 13,400 g) for 10 min at 4°C. Remove the supernatant from the tube, leaving only the RNA pellet. The RNA is often invisible prior to centrifugation and forms a gel-like pellet on the side and bottom of the tube.
8. Wash the pellet with at least 1 mL of 75% ethanol (in Rnase-free ddH_2O) for every 1 mL of TOOLSmart RNA extractor used in the initial homogenization.
9. Centrifuge the tube at 10,000 rpm (approximately 9,391 g) for 5 min at 4°C. Discard the supernatant. Centrifuge briefly to collect the residual liquid and then remove it without aspirating the pellet.

10. Air dry the RNA pellet for 2–3 min. Do not allow the RNA to dry completely because the pellet can lose solubility. According to the requirements, add 30–100 μL of RNase-free ddH_2O and resuspend the RNA pellet completely by repeatedly passing the solution up and down through a pipette tip.

Expected Yields

The following table presents typical yields of RNA from various starting materials.

Starting Material	**RNA Yield**
Plant leaf	100-500 μg / 1 g leaves
Animal tissue	6-10 μg / 1 mg liver
Cultured cell	5-10 μg / 1 × 10^6 cells
Blood	3-5 μg / 1 mL human whole blood