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TECHNICAL DATA SHEET

NAPzol RNA Isolation Reagent

AM1019-1-100 ML, AM1019-1-200 ML

PRODUCT OVERVIEW

NAPzol is a ready-to-use reagent designed to isolate high-quality total RNA from human, animal, plant, yeast, or bacterial cell and tissue samples within one hour. This monophasic solution contains phenol, guanidine salts, and proprietary components that facilitate the isolation of various RNA species, including both large and small molecules. NAPzol preserves RNA integrity by effectively inhibiting RNase activity while disrupting cells and dissolving cell components during homogenization. It enables simultaneous processing of multiple samples and enhances the single-step RNA isolation method.

The NAPzol reagent enables users to isolate RNA, DNA, and proteins sequentially from a single sample. After homogenizing the sample with NAPzol, chloroform is added. The mixture then separates into three layers: a clear upper aqueous layer, an interphase, and a lower red organic layer. RNA is precipitated from the aqueous layer using isopropanol, while DNA is precipitated from the interphase/organic layer using ethanol. Proteins are precipitated from the phenol-ethanol supernatant using isopropanol. The precipitated RNA, DNA, or protein is then washed to remove impurities and resuspended for downstream applications.

STORAGE CONDITIONS

NAPzol RNA Isolation Reagent

NAPzol is shipped at room temperature but should be stored at 2–8°C.

TO BE SUPPLIED BY THE USER

- Equipment: Centrifuge and rotor capable of reaching 12,000 × g and 4°C, Water bath or heat block at 55–60°C.
- Sterile tubes: 1.5 ml polypropylene microcentrifuge tubes.
- Reagents: Chloroform, Isopropanol, Ethanol, RNase-free water, RNase-free glycogen (optional), or 0.1 mM EDTA (Optional).

GUIDELINES FOR PROCEDURES

- Unless otherwise specified, carry out all steps at room temperature (20–25°C).
- We recommend using chilled NAPzol reagent for all samples, especially when working with starting materials with high RNase levels, such as spleen or pancreas tissue.
- Use disposable sterile plasticware, RNA-free disposable pipettes, pipette tips, and tubes.
- Wear disposable gloves when handling reagents and RNA samples to prevent RNase contamination from your skin. Always apply proper microbiological aseptic techniques when handling RNA.
- Ensure all materials that come into contact with NAPzol Reagent are compatible with phenol, guanidine salts, and chloroform.

INPUT SAMPLE REQUIREMENTS

To preserve RNA integrity, immediately isolate RNA after sample collection. If this isn't possible, quickly freeze the samples using snap freezing in liquid nitrogen or dry ice. Store frozen samples at -80°C to prevent RNA degradation, and for long-term storage, liquid nitrogen is recommended. Additionally, RNA stabilizing reagents can be used immediately after sample collection to inhibit RNase activity. Alternatively, tissue homogenized in NAPzol Reagent or cells resuspended in NAPzol Reagent can be stored at –80°C for up to a year. Avoid repeated freeze-thaw cycles by aliquoting samples before freezing. The amount of starting solid tissue or the number of cells can be selected based on the information in the table below.

Sample type	Amount of starting material per 1 ml of NAPzol Reagent.
Animal/plant tissues	40-120 mg of tissue
Cells grown in a monolayer/suspension	1-10x10^6 cells grown in a monolayer
Bacterial cells	5-10x10^7
Yeast cells	5-10x10^6
Biological fluids	<250 µl
Blood	Upto 300 µl

PROCEDURE

A. LYSE THE SAMPLES AND SEPARATE THE PHASES

1. Lyse and homogenise samples based on the starting material given below.
   - **a. Cells grown in a monolayer**
     a. Remove growth media, and add 1 ml of NAPzol Reagent per $1x10^5$–$1x10^7$ cells directly to the culture dish to lyse the cells.
     b. Pipette the lysate up and down several times to detach and homogenise the cells.

   - **b. Cells grown in suspension**
     a. Collect the cells by centrifuging at 500 *x g* for 5 min and discard the supernatant. ***Note:*** Avoid washing the cells before adding NAPzol Reagent to prevent RNA degradation.
     b. Add 1 ml of NAPzol Reagent per $1x10^5$- $1x10^7$ cultured animal cells, pipette the lysate up and down multiple times to homogenize.

   - **c. Bacteria and yeast**
     a. Transfer 1.5 mL of bacterial culture (~1 × 10^8 cells) or yeast culture (~1 × 107 cells) into a microcentrifuge tube.
     b. Centrifuge at 6,000 × g for 5 minutes at 4 °C to pellet the cells. In the meantime, preheat the required volume of Lyse Enhancement Reagent.
     c. Discard the supernatant and resuspend the cell pellet in the preheated 200 µL Lyse Enhancement Reagent, mixing thoroughly by pipetting.
     d. Incubate the suspension at 95 °C for 4-5 minutes. Do not exceed the heating time for more than 5 min.
     e. Add 1 ml of NAPzol Reagent to the lysate, and pipette the lysate up and down multiple times to homogenize.

   - **d. Animal/Plant tissues**
     a. Add 1 ml of NAPzol Reagent per 40–120 mg of tissue to the sample and grind in a mortar/pestle, dounce, syringe, tissue grinder, or homogeniser.
     b. Pipette the lysate up and down multiple times to homogenize.

   - **e. Biological fluids**
     a. Add 1 ml of NAPzol Reagent to up to 250 µl of biological liquid. ***Note:*** The volume of the sample should be at most 25% of the volume of NAPzol Reagent used for lysis.
     b. Pipette the lysate up and down multiple times to homogenize.

   - **f. Blood**
     a. Take up to 300 µl of blood in a 2 ml tube and add 1.5 ml of RBC Lysis Buffer (not supplied, purchased separately), then pulse vortex or invert to mix, and incubate for 10-15 min at ice.
     b. Mix or pulse vortex again and centrifuge at 500 × g for 5 minutes at 4°C. Discard supernatant.

c. Add 1 ml of NAPzol Reagent to the pellet.
d. Pipette the lysate up and down multiple times to homogenize.

2. Incubate for 5 minutes at room temperature to ensure complete lysis and dissociation of the nucleoprotein complex.
3. ***(Optional)*** If the samples are high-fat, centrifuge the lysate at 12,000 × g for 5 minutes at 4–10°C, then transfer the clear supernatant to a new tube.
4. Add 0.2 mL of chloroform per 1 mL of NAPzol Reagent used for lysis, securely cap the tube, and then mix thoroughly by shaking.
5. Incubate for 3 minutes at room temperature.
6. Centrifuge the sample for 12 minutes at 12,000 × g and 4°C. The mixture will separate into a lower red phenol-chloroform phase, an interphase, and a colorless upper aqueous phase.
7. Transfer ~80% of the colorless upper phase containing the RNA to a new tube. ***Note:*** When removing the aqueous phase, do not transfer any interphase or organic layers into the pipette.

B. ISOLATE RNA FROM THE AQUEOUS PHASE

1. (**Optional**) If the starting sample is small (<10^6 cells or 10 mg of tissue), add 6–12 µg of RNase-free glycogen as a carrier to the aqueous phase. Glycogen co-precipitates with the RNA but does not interfere with subsequent applications.
2. Add an equal volume of isopropanol to the aqueous phase and incubate at 4°C or ice for 10 minutes (i.e., 500 µl of isopropanol per 500 µl of aqueous phase).
3. Centrifuge for 10 minutes at 12,000 × g at 4°C. The total RNA precipitate forms a white, gel-like pellet at the bottom of the tube. Carefully discard the supernatant without disturbing the pellet.
4. Add 750 µl of 75% ethanol, and invert to mix and centrifuge for 5 minutes at 7500 × g and 4°C. ***Note:*** RNA can be stored in 75% ethanol at –20°C for up to 1 year or at 4°C for up to 1 week.
5. Carefully discard the supernatant without disturbing the pellet, and air-dry the RNA pellet for 5 to 10 minutes. Be sure the RNA pellet does not dry out completely to ensure complete solubilization.
6. Resuspend the pellet in 20-100 µl of RNase-free water, 0.1 mM EDTA, or 0.5% SDS, depending upon the amount of starting sample, and mix by pipetting up and down.
7. Incubate at ice for 10–15 minutes while flicking every 2-3 minutes to facilitate the solubilization of the RNA. ***Note:*** If the RNA pellet is overdried or difficult to dissolve, briefly heating the tube at 55-60°C for 10-15 minutes can help facilitate RNA solubilization.
8. Proceed with downstream applications or store the RNA at –70°C.

Measure the RNA yield.

To measure RNA yield, you can use absorbance or fluorescence methods. For absorbance, measure at 260 nm to determine total nucleic acid content and at 280 nm to assess sample purity. Dilute the sample in RNase-free water and calculate RNA concentration using A260 × dilution factor × 40 = µg /ml RNA. The A260/A280 ratio, ideally around 2, indicates purity. Alternatively, you can quantify RNA directly without dilution using a NanoDrop™ Spectrophotometer. The fluorescence method, which selectively measures intact RNA, does not detect proteins or other contaminants, providing a more specific RNA quantification.