NZYol

Catalogue number: MB18501, 100 mL

Features

- Quick isolation of high-quality RNA
- Ready-to-use solution for a wide variety of cells and tissues, including animal and plant tissues rich in polysaccharides and peptidoglycans
- Cost-effective
- 1 hour protocol
- Convenient and reliable
- Pure RNAs is ideal for any downstream applications, including RT-PCR and *in vitro* translation

Description

NZYol is a ready-to-use reagent combining a blend of phenol and other components for the isolation of high-quality total RNA (as well as DNA and protein) from cell and tissue samples. NZYol disrupts cells exposing cellular components and maintains the integrity of the extracted RNA. Biological samples are homogenized or lysed in NZYol and then separated into three phases: a clear aqueous phase (upper) containing the RNA, a green organic phase (lower) and an interphase, containing DNA and protein, respectively. RNA is recovered by precipitation with isopropyl alcohol. DNA is precipitated from the organic layer with ethanol and the proteins are precipitated from the phenol-ethanol supernatant by isopropyl alcohol precipitation. The precipitated RNA, DNA, or protein are subsequently washed to remove impurities, and then resuspended for use in downstream applications. Isolated RNA is suitable for any downstream application such as RT-PCR, hybridization assays, or *in vitro* translation. 1 mL of NZYol is sufficient to isolate RNA and DNA from 1 x 10^7 cells or 100 mg of tissue.

Storage conditions

Store NZYol at 4 °C. The reagent maybe stored at room temperature for short periods of time.

Safety Precautions

Toxic in contact with skin. Toxic if swallowed. Causes burns. Please refer to the material safety data sheet information regarding hazards and safe handling practice.

Protocol for the isolation of Total RNA using NZYol

Perform RNA isolation immediately after sample collection or quick-freeze samples immediately after collection until RNA isolation.

1. Lysis and homogenization

Tissue: Homogenize tissue samples in 1 mL of NZYol per 50-100 mg of tissue.

Notes: *For small quantities of tissue (1-10 mg), add 800 µL of NZYol. If samples have a high fat content, a layer of fat may accumulate at the top, which should be removed by centrifugation for 5 minutes at* 12,000 *xg at 4-10°C.*

Plant tissue: Add 1 mL of NZYol to the sample and homogenize. After homogenization, remove insoluble material by centrifugation at 12,000 *xg* for 10 min, at 4 °C. Transfer the cleared homogenate to a fresh tube.

Cells grown on monolayer: Remove growth media and lyse cells directly in a culture dish or flask by adding 1 mL of NZYol per 10 cm^2 growth area. Pipette the cell lysate several times to ensure sufficient cell disruption.

Cells grown in suspension: Pellet cells by centrifugation at 200 *xg* for 5 min, at room temperature. Add 1 mL of NZYol per 5 x 10^6 cells to the pellet. Pipette the lysate up and down several times to homogenize.

Note: *For small quantities of cells (10^2-10^6), lyse cells in 800 µL of NZYol.*

At this stage, samples can be stored at 4 °C overnight or at -70 °C for up to a year.

2. Phase Separation

2.1. Incubate samples for 5 min at room temperature.

2.2. Add 0.2 mL of chloroform per 1 mL of NZYol used. Cap tubes securely and shake vigorously by hand for 15 seconds.

2.3. Incubate samples for 2-3 min at room temperature.

2.4. Centrifuge samples at 12,000 *xg* for 15 min at 4 °C. The sample will separate into a lower green phenol-chloroform phase, an interphase, and a colourless upper aqueous phase that contains the RNA.

2.5. Transfer the aqueous phase very carefully, without disturbing the interphase or organic layer, to a new tube.

Save the interphase or organic phase if you want to isolate DNA or protein, respectively. The interphase and organic phase can be stored overnight at 4°C. See below "Protocol for the isolation of DNA using NZYol" and "Protocol for the isolation of proteins using NZYol" for detailed procedures.

3. RNA Precipitation

3.1. Precipitate the RNA by mixing with cold isopropyl alcohol. Add 0.5 mL of isopropyl alcohol to the aqueous phase, per 1 mL of NZYol used for lysis.

3.2. Incubate samples for 10 min at -20 ºC.

3.3. Centrifuge at 12,000 *xg* for 10 min at 4 °C. Discard the supernatant with a micropipette.

Note: *Total RNA precipitate forms a white gel-like pellet at the bottom of the tube.*

4. RNA Wash

4.1. Resuspend the pellet in 1 mL of 75% ethanol, per 1 mL of NZYol used for lysis.

4.2. Mix samples briefly, and then centrifuge at 12,000 *xg* for 5 min at 4 °C. Discard the supernatant with a micropipette.

4.3. Air dry the pellet for 5-10 min.

At this stage, samples can be stored for one week at 4 °C, or 12 months at -5 to -20 °C.

5. Re-dissolving the RNA

5.1. Gently resuspend the pellet in RNase-free water or DEPC-treated water by pipetting the solution up and down.

5.2. Incubate for 10 min at 55-60 °C if necessary.

5.3. Store RNA at -70 °C.

Protocol for the isolation of DNA using NZYol

1. DNA Precipitation

1.1. Remove any remaining aqueous phase overlying the interphase.

1.2. Add 0.3 mL of 100% Ethanol, per 1 mL of NZYol used for lysis. Cap tubes securely and shake vigorously by hand for 15 seconds.

1.3. Incubate samples for 2-3 min at room temperature, then centrifuge at 12,000 *xg* for 5 min at 4°C to pellet the DNA. Remove the supernatant to waste or retain for protein isolation.

2. DNA Wash

2.1. Wash the DNA pellet with 1 mL of 0.1 M sodium citrate in 10% ethanol, pH 8.5, per 1 mL of NZYol used for lysis.

2.2. Incubate for 30 min at room temperature, mixing occasionally.

2.3. Centrifuge at 2000 *xg* for 5 min at 4°C.

2.4. Repeat step 2.1-2.3 once.

Note: *Two washes are usually sufficient. However, for large DNA pellets (>200µg) an additional wash may be necessary.*

2.5. Following the washing, resuspend the pellet in 1.5 mL of 75% ethanol, per 1 mL of NZYol used for lysis.

2.6. Incubate for 20 min at room temperature, mixing occasionally.

2.7. Centrifuge at 12,000 xg for 5 min at 4°C. Discard the supernatant with a micropipette.

2.8. Air-dry the pellet for 10-15 min.

3. Re-dissolving the DNA

3.1. Gently resuspend the pellet in 8 mM NaOH by pipetting the solution up and down.

3.2. Remove any insoluble material by centrifugation at 12,000 *xg* for 10 min at 4°C, and then transfer the supernatant to a new tube.

3.3. Samples can be stored overnight at 4°C. For long-term storage at -20°C, adjust the pH to 7.5 with HEPES and adjust the EDTA concentration to 1 mM.

Protocol for the isolation of Proteins using NZYol

1. Protein Precipitation

1.1. Add 1.5 mL of isopropyl alcohol to the retained phenol-ethanol supernatant (step 1.3 of the DNA isolation protocol), per 1 mL of NZYol used for lysis.

1.2. Incubate for 5 min at room temperature, then centrifuge at 12,000 *xg* for 10 min at 4°C. Discard the supernatant with a micropipette.

2. Protein Wash

2.1. Remove supernatant and wash the protein pellet twice. Prepare a wash solution of 0.3 M guanidine hydrochloride in 95% ethanol.

2.2. Add 2 mL of wash solution, per 1 mL of NZYol used for lysis.

2.3. Incubate for 20 min at room temperature, then centrifuge at 7500 *xg* for 5 min at 4°C. Discard the supernatant with a micropipette.

At this stage, samples can be stored in wash solution for at least one month at 4°C, or 12 months at -20°C.

2.4. Add 2 mL of 100% ethanol, then vortex briefly.

2.5. Incubate for 20 min at room temperature, then centrifuge at 7500 *xg* for 5 min at 4°C. Discard the supernatant with a micropipette.

2.6. Air dry the protein pellet for 5-10 min.

3. Re-dissolving the Protein

3.1. Resuspend the pellet in 1% SDS by pipetting up and down.

Note: *For difficult samples incubate at 50°C.*

3.2. Centrifuge at 10,000 *xg* for 10 min at 4°C to remove insoluble materials. Transfer the supernatant to a new tube.

3.3. Store protein at -20°C.

Troubleshooting

Genomic DNA contamination
• Insufficient volume of NZYol used
Ensure the usage of the recommended volumes of NZYol.
• Incomplete Lysis or homogenization
Homogenize tissue thoroughly and centrifuge to remove insoluble material.
• Contamination of interphase layer during RNA separation
Pipette off the aqueous phase very carefully.
RNA degradation
• RNase contamination
The protocol must be carried out carefully in a DNA-free, RNase-free environment.
Low RNA yield
• Loss of pellet
If starting sample is small, the RNA pellet may not be easily visualized after isopropyl alcohol precipitation, so care must be taken when removing the supernatant from the pellet
• Incomplete lysis or homogenization
Homogenize tissue thoroughly and centrifuge to remove insoluble material. Pipette resultant cell lysate up and down thoroughly until it becomes visually less viscous

Quality control assays

Functional assay

NZYol is tested following the nucleic acid isolation protocols described above. The purification system must isolate pure total RNA with high integrity.

V1901

Certificate of Analysis

Test	Result
Functional assay	Pass

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems