Technical Data Sheet

Xtrazol Reagent

for molecular biology

Order number: 2348

Quick isolation of high-quality total RNA, DNA and/or protein from a single sample.

Xtrazol Reagent is a ready-to-use reagent for the isolation of total RNA/DNA/protein from various biological materials such as animal and plant tissues (rich in polysaccharides and proteoglycans), cell culture and bacterial cells. The biological sample is homogenized or lyzed with Xtrazol Reagent before it is separated into three phases: an aqueous phase (upper), an organic phase (lower) and an interphase. The isolation method is fast and easy to carry out.

The RNA remains in the aqueous phase and can be isolated by precipitation in 2-Propanol. The highly effective RNase inhibitory property of Xtrazol Reagent protects the integrity of the RNA during lysis and results in the isolation of high-quality material (high RNA Integrity Number and S28/S18 rRNA values).

One ml of Xtrazol Reagent is sufficient to isolate total RNA from 1 x 10^7 cells or 100 mg of tissue. RNA obtained by using Xtrazol Reagent provides reliable results in gene expression-based studies using methods such as synthesis of cDNA, real-time RT-PCR, microarrays, hybridization assays, and in vitro translation.

DNA can be precipitated and purified from the interphase/organic phase and subsequently applied for PCR and Southern blotting. Proteins purified with Xtrazol Reagent are suitable for Western blotting.

Xtrazol Reagent is a monophasic mixture containing Guanidine thiocyanate and Phenol. It is an improved version of the single-step total RNA isolation reagent developed by Chomczynski[1].

Reagents required but not supplied with Xtrazol Reagent

Description	neoFroxx Product Code	Volume
Chloroform p.A., stabilized with 50 ppm Amylene	LC-4919.4	250 ml
2-Propanol for molecular biology	1496LT001	1 L
Ethanol absolute for molecular biology	1135LT001	1 L
Water for molecular biology (RNase-free)	1058LT001	1 L
tri-Sodium citrate dihydrate for molecular biology	1795GR500	500 g
Sodium hydroxide pellets for molecular biology	1808GR500	500 g
EDTA for molecular biology	1340GR100	100 g
EDTA disodium salt dihydrate for molecular biology	1108GR500	500 g
Guanidine hydrochloride for molecular biology	1324GR100	100 g
SDS solution 10 % for molecular biology	1473LT001	1 L

[1] Chomczynski, P. (1993) BioTechniques 15, 532-537: A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples.

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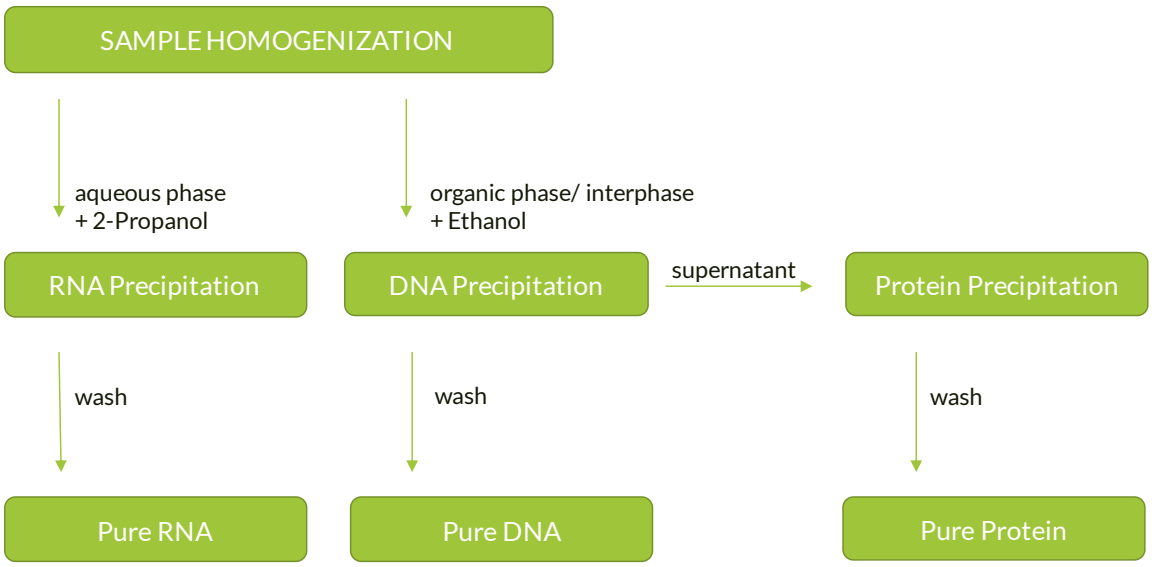
Protocol



A) HOMOGENIZATION

- **Tissue:** Homogenize tissue samples in 1 ml of Xtrazol Reagent per 50-100 mg of tissue. For small quantities of tissue (1-10mg), add 800 µl of Xtrazol Reagent. For samples of fat tissue, a layer of fat may accumulate at the top, which should be removed.
- **Plant tissue:** Following homogenization, insoluble material is removed by centrifugation at 12000 x g for 10 minutes at 4°C. Transfer the cleared homogenate to a fresh tube.
- **Cells grown on monolayer:** Lyse cells directly in a culture dish or flask by adding 1 ml of Xtrazol Reagent per 10 cm^2 growth area, pipette the cell lysate several times to ensure sufficient cell disruption.
- **Cells grown in suspension:** Pellet cells at 200 x g for 5 minutes at room temperature. Lyse cells with 1 ml of Xtrazol Reagent per 5 x 10^6 cells and pass the lysate several times through a pipette tip. For small quantities of cells (10^2 – 10^6), lyse cells in 800 µl of Xtrazol Reagent.

Note: at this stage, samples can be stored for at least one month at -70°C.

B) PHASE SEPARATION

1. Incubate samples for 5 minutes at room temperature.
2. Add 0.2 ml of Chloroform or 1-Bromo-3-chloropropane per 1 ml of Xtrazol Reagent.
3. Cap tubes securely and shake vigorously by hand for 15 seconds.
4. Incubate samples for 3-10 minutes at room temperature.
5. Centrifuge samples at 12000 x g for 15 minutes (or 2600 x g for 30 minutes) at 4°C.
6. The sample will separate into a pale-yellow organic phase, an interphase and a colorless upper aqueous phase that contains the RNA.

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C) ISOLATION OF RNA: AQUEOUS PHASE

I. RNA Precipitation

1. Transfer the aqueous phase very carefully to another reaction tube, without disturbing the interphase. **Keep the organic phase/interphase containing the DNA/protein at +4°C.**
2. Precipitate the RNA by mixing with cold 2-Propanol. Use 0.5 ml of 2-Propanol per 1 ml of Xtrazol Reagent[#].
3. Incubate sample for 10 minutes at room temperature.
4. Centrifuge at 12000 x g for 10 minutes (or 2600 x g for 30 minutes) at 4°C.

II. RNA Purification

1. Remove the supernatant.
2. Wash the RNA-pellet once with 75% Ethanol, adding at least 1 ml of Ethanol per 1 ml of Xtrazol Reagent[#].
3. Vortex samples and centrifuge at 7500 x g for 5 minutes at 4°C.

III. Re-dissolving the RNA

1. Air-dry the pellet and dissolve in RNase-free water by pipetting the solution up and down. Do not let the RNA pellet dry out completely, as this will decrease its solubility!
2. Incubate for 10 minutes at 60°C if necessary.
3. Store RNA at -70°C.

D) ISOLATION OF DNA: INTERPHASE AND ORGANIC PHASE

I. DNA Precipitation

1. Completely remove the residual aqueous phase from the interphase and organic phase to improve the purity of the DNA.
5. Selectively precipitate the DNA by mixing the interphase/organic phase with cold Ethanol. Add 0.3 ml Ethanol per 1 ml of Xtrazol Reagent[#].
2. Incubate mixture for approx. 5 minutes at room temperature.
3. Centrifuge at 2.000 g for 5 minutes at +4°C.

II. DNA Purification

1. Remove the supernatant (Ethanol/Phenol phase). **Keep the supernatant at +4°C for the subsequent protein purification.**

2. Wash the DNA precipitate in 1 ml 0.1 M Sodium citrate/10 % Ethanol per 1 ml Xtrazol Reagent[#]
3. Incubate at least for 30 minutes at room temperature mixing occasionally.
4. Centrifuge at 2.000 g for 5 minutes at room temperature.
5. Repeat steps 2. - 4.

[#] Based on the Xtrazol volume originally used.

III. Re-dissolving the DNA

1. Air-dry the DNA and dissolve in 8 mM NaOH by pipetting carefully with a micropipette. This mild alkaline solution assures complete dissolution of the DNA pellet. Samples dissolved in 8 mM NaOH can be stored at 2–8 °C overnight.
2. Sediment remaining cell fragments by centrifugation at 12.000 g for 10 minutes.
3. Transfer the DNA-containing supernatant to a new reaction tube. For long term storage, adjust the pH value to between 7 and 8 and supplement with EDTA (final concentration 1 mM).

E) ISOLATION OF PROTEINS: ETHANOL/PHENOL PHASE

I. Protein Precipitation

1. Use Phenol/Ethanol phase from DNA isolation D) II. 1. for protein precipitation.
2. Add twice the sample volume of 2-Propanol. Proteins will precipitate during an incubation of approx. 10 minutes at room temperature.
3. Centrifuge at 12.000 g for 10 minutes at +4°C.

II. Protein Purification

1. Wash the protein precipitate with 2 ml of 300 mM Guanidine hydrochloride in 95 % Ethanol per 1 ml Xtrazol Reagent[#].
2. Incubate for 20 minutes at room temperature.
3. Centrifuge at 7.500 g for 5 minutes at +4°C.
4. Repeat steps 1. - 3. twice.

III. Re-dissolving the proteins

1. Air-dry or vacuum-dry the protein precipitate and dissolve it in 1 % SDS. Warming up to 50°C might be necessary.
2. If you detect residual cell fragments or other insoluble components, remove them by an additional centrifugation step at 10.000 g for 10 minutes at +4°C.
3. Transfer the supernatant to a new reaction tube and store at -20°C.

Storage

Store Xtrazol Reagent at 2-8°C.

[#] Based on the Xtrazol volume originally used.

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