

Manual

Fenzol

Fenzol is chaotropic salts and phenol mixture dedicated to RNA isolation. Fenzol deactivates endogenous RNAses.

catalog #	size
203-50	50 ml
203-100	100 ml

For research use only.

Guarantee

A&A Biotechnology provides guarantee on this product.

The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

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Description

- Fenzol is a chaotropic salts and phenol mixture dedicated to RNA isolation.
- Fenzol deactivates endogenous RNAses.

Sample suspended in fenzol can be stored at:

- -20 °C to -80 °C: up to one year.
- +4 °C to +8 °C: up to six months.
- room temperature: up to one week.

Procedural guidelines

- Perform all steps at room temperature unless otherwise noted.
- Use cold fenzol if the starting material contains high levels of RNase, such as spleen or pancreas samples.
- Use disposable, individually wrapped, sterile plastic ware and sterile, disposable RNase-free pipettes, pipette tips, and tubes.
- Wear disposable gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin; change gloves frequently, particularly as the protocol progresses from crude extracts to more purified materials.
- Always use proper microbiological aseptic techniques when working with RNA.

Material preparation

1. Lyse and homogenize samples in fenzol according to your starting material:

Tissues

- A. Add 1 ml of **fenzol** per **100 mg** of **tissue** and homogenize using a suitable homogenizer.

Tissue culture cells in suspension

- A. Pellet the cells by centrifugation and discard the supernatant.
- B. Add 1 ml of **fenzol** per **250 µl** of **sample** (**5–10×10⁶ cells from animal, plant or yeast origin or 1×10⁷ cells of bacterial origin**) to the pellet.
Note: Do not wash cells before addition of fenzol to avoid mRNA degradation.
- C. Pipet the lysate up and down several times to homogenize.

Note: The sample volume should not exceed 10% of the volume of fenzol used for lysis.

Samples can be stored at **4 °C overnight** or at **-20 °C up to one year**.

Option: If sample has a high fat content, centrifuge the lysate for **5 min** at **12 000 × g** at **4–10 °C**. Transfer the clear supernatant to a new tube.

Incubate for **5 min** to permit complete dissociation of the nucleoproteins complex.

2. Add **200 µl** of **chloroform** per **1 ml** of **fenzol** used for lysis, close the tube with the cap. Mix the sample by inverting the tube to efficiently mix the solution phases. Incubate for **2–3 min**. Centrifuge for **15 min** at **12 000 × g** at **4 °C**.

The mixture separates into a lower brownish phenol-chloroform and interphase and colourless upper aqueous phase.

3. Transfer the aqueous phase containing RNA to a new tube by angling the tube at 45° and pipetting the solution out.

IMPORTANT! Avoid transferring any of the interphase or organic layer into the pipette when removing the aqueous phase.

Protocol

Option: If the sample is small (<10⁶ cells or <10 mg of tissue), add 5-10 µl of precipitation enhancer (cat. # 197-1) or 5 µg polyA RNA as a carrier to the aqueous phase.

1. Add equal volume of isopropanol to the aqueous phase (usually 450-500 µl of isopropanol per 1 ml of fenzol used for lysis).
3. Mix the sample by inverting the tube a few times. Incubate for 3 min at room temp.
4. Centrifuge for 10 min at 12 000 x g at 4 °C.
Total RNA precipitate forms a white gel-like pellet at the bottom of the tube.
5. Discard supernatant with a micropipette.
6. Resuspend the pellet in 1 ml of 75% ethanol per 1 ml of fenzol used for lysis.
Note: RNA in 75% ethanol can be stored: at -20 °C up to one year, at -4 °C up to one week.
7. Briefly vortex the sample and centrifuge for 5 min at 7500 x g at 4 °C.
8. Discard supernatant with a micropipette. Vacuum or air dry RNA pellet for 5-10 min at room temp.

IMPORTANT!

Do not dry the pellet by vacuum centrifuge. Do not let the RNA pellet overdry, to ensure total solubilization of the RNA. Partially dissolved RNA samples have an $A_{230/280}$ ratio <1.6

9. Resuspend the pellet in 20-50 µl of RNase-free water (cat. # 003-075, 003-25) or 0.1 mM EDTA.
10. Incubate in a water bath or heat block set for 10-15 min at max 55 °C.
11. Follow the next application or store RNA samples at -70 °C.

Safety information



DANGER

H301+H311+H331 Toxic if swallowed, in contact with skin or if inhaled.

H314 Causes severe skin burns and eye damage.

H341 Suspected of causing genetic defects.

H373 May cause damage to organs through prolonged or repeated exposure.

H411 Toxic to aquatic life with long lasting effects.

P261 Avoid breathing dust.

P273 Avoid release to the environment.

P280 Wear protective gloves, protective clothing, eye protection, face protection.

P301+P310 If swallowed: immediately call a Poison Center or doctor/physician.

P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310 Immediately call a Poison Center or doctor/physician.



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