A&A BIOTECHNOLOGY
innovating life science

Manual

Fenozol Plus

Fenozol Plus is a chaotropic salts and phenol mixture dedicated to RNA isolation. Fenozol deactivates endogenous RNAses.

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

- Fenozol Plus is a chaotropic salt and phenol mixture dedicated to RNA isolation.
- Fenozol Plus deactivates endogenous RNAses.
- Store at 2-8 ℃.

Sample suspended in fenozol plus can be stored at:

- -20 ℃ to -80 ℃: up to one year.
- 2-8 ℃: up to six months.
- room temperature: up to one week.

Procedural guidelines

- Perform all steps at room temperature unless otherwise noted.
- Use cold fenozol plus 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 fenozol plus according to your starting material:

   Tissues

   A. Add **1 ml** of **fenozol Plus** 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 **fenozol Plus** per **250 µl** of **sample (5–10×10^6 cells from animal, plant or yeast origin or 1×10^7 cells of bacterial origin**) to the pellet.
   Note: Do not wash cells before addition of fenozol plus 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 fenozol Plus 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 **fenozol Plus** 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 x 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 (<**10^6 cells** or **<10 mg of tissue),** add **5-10 µl** of precipitation enhancer (cat. # K-WZM-12) 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 fenozol plus 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 m**l of **75% ethanol** per 1 ml of fenozol Plus 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 7**500 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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