Instructions for use

ROTI®Zol RNA

Ready-made solution with green dye for isolation of total RNA.

- Easy and very rapid two-step RNA isolation
- High recovery rates of intact, very pure total RNA
- Applicable on a broad range of tissues and on cell types
- The purified RNA is ready for use in standard downstream applications such as RT-PCR.

ROTI®Zol RNA is a single phase phenol solution that is used for the isolation of total RNA from a variety of cell and tissue types.
ROTI®Zol RNA is based on the phenolic isolation procedure known from Chomczynski and Sacchi. Since the tissue is homogenised directly in ROTI®Zol RNA, RNase activity is immediately inhibited, preventing degradation of all classes of RNA. RNA isolation in ROTI®Zol RNA includes homogenization, phase/molecule separation, and RNA precipitation.

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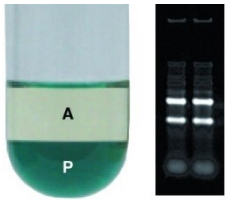
Due to the advanced and optimized formulation, ROTI®Zol RNA results in very high recovery rates of highly pure total RNA. Additionally, the green colour significantly simplifies the separation of the RNA containing aqueous phase, the DNA containing interphase, and the protein containing phenolic phase during the process.

Typical yield:
Typical RNA yield
from $5x10^6$ cells: 30-35 µg
Typical purity of RNA prepared
from $5x10^6$ cells: 2.08 (A_{260}/A_{280})

1 ml ROTI®Zol RNA is used for RNA isolation from 100 mg tissue or 10^7 cells, respectively.

Application
Isolation procedure is done in less than one hour. Please make sure that from step 8 on, only RNAse-free material (tubes, pipet tips etc.) and reagents is used.

1. Introduce sample (5-10×10^6 cells, 50-100 mg tissue) into a reaction tube.
2. Add 1 ml ROTI®Zol RNA.
3. Homogenize tissue sample in ROTI®Zol RNA by a glass-Teflon® or powder homogenizer. Cells from cell culture may be lysed by pipetting.
   Note: make sure the homogenization technique is adequate for the respective sample. Thorough homogenization is vital for recovery of the RNA.
4. Incubate at room temperature (RT) for 5 mins.
5. Add 200 µl chloroform and shake or vortex the tube for a few seconds.
6. Incubate at RT for 3 mins.
7. Centrifuge the tube at 12.000 x g for 15 mins. at +4 °C.
8. Transfer the upper, unstained, aqueous, phase to a fresh, RNAse-free tube.
9. Discard the lower, blue-green stained, phenolic phase.
10. Add 500 µl 2-propanol and incubate at RT for 10 mins.
11. Centrifuge the tube at 12.000 x g for 10 mins. at +4 °C for precipitation of the RNA.
12. Remove the supernatant (approx. 1 ml).
13. Add approx. 1.5 ml 75 % cold ethanol and shake or vortex the tube briefly.
14. Centrifuge the tube at 12.000 x g for 5 mins. at +4 °C for precipitation of the RNA pellet.
15. Remove the supernatant (approx. 1 ml).
16. Let the residual ethanol briefly evaporate at RT.
17. Resolubilise the RNA pellet in RNAse-free water.



Example for isolation of totalRNA by ROTI®Zol RNA. Left: Tube after centrifugation. A: Aqueous phase - RNA containing, P: Phenolic phase - protein containing. DNA accumulates in the A/P interphase.
Right: Typical gel of total RNA isolated by ROTI®Zol RNA (extracted from $5x10^6$ HL-60 cells).

Further Recommended Products:
Water, RNAse-free, BioScience-Grade (Art. No. T143)
DEPC (Art. No. K028)
Ethanol ≥99,8 %, p.a. (Art. No. 9065)
2-Propanol ≥99,8 %, p.a., ACS, ISO (Art. No. 6752)

Reference
Chomczynski and Sacchi (1987) *Anal. Biochem.* 162:156-159.

Storage:
Store at +4 °C. Do not freeze!
Purified total RNA should be stored at -80 °C.

Danger
H290-H302+H312+H332-H314-H341-H373-H411-EUH032-EUH208

ROTI®Zol RNA	
9319.1	100 ml
9319.2	200 ml

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