

RNA Extraction Kits

Total Quick RNA

Total Quick RNA is a kit designed for RNA mini extractions (up to 100 µg). The kit has a short processing time (20 minutes) and its simple procedure allows multiple samples to be processed in the *same* run. The protocol is common to all starting substrates, apart from those (bacteria) which require an enzymatic incubation. The process employs common laboratory equipment.

Total Quick RNA: Principle and Procedure

The Total Quick RNA kit technology combines the speed of a spin column process with the advantage of a detergent-based chemistry. The detergent in the lysing solution lyses the cells and precipitates nucleic acids (both DNA and RNA) as complexes with the surfactant. The RNA is trapped in a reverse micelle and is consequently protected from ribonuclease activity.

After gentle homogenisation, the lysate is transferred to the spin column, incubated for a few minutes at 4°C and then centrifuged. This process eliminates the liquid phase and allows the retention of the detergent-nucleic acid complexes on the spin column filter.

Addition of the washing solution dissociates detergent-nucleic acids complexes and solubilises the DNA, which is eliminated together with any trace proteins after a second spin. The RNA which remains on the filter is washed with 70 % ethanol, resuspended in water and recovered with a short centrifugation. The RNA purified using Total Quick RNA is suitable for RT-PCR and c-DNA synthesis.

Components of the kit

- Total Quick Spin Columns, 20 pieces
- 2 ml Collection tubes, 20 pieces
- 1,5 ml Collection Tubes, 20 pieces
- Lysing Solution, 15 ml
- Washing Solution, 13 ml
- RNase-free water, 2 ml
- Handbook

Reagents and Equipment required

- absolute ethanol (7 ml to be added to the washing solution before use)
- **70 %** ethanol in water
- sterile RNase-free pipette tips
- refrigerated microcentrifuge with rotor for 2 ml tubes (maximum speed required: 10.000 RPM)
- disposable gloves
- lysozyme, 20 mg/ml in water (for bacterial RNA extractions only)
- TE-buffer, pH 8,0 (for bacterial RNA extractions only)

General Remarks

- It is important that contamination of the glassware, plasticware (tubes, tips, etc.) solutions and reagents used for RNA extraction is eliminated.
- Gloves must be worn to prevent RNase contamination when handling reagents and RNA samples.
- All the steps of the Total Quick RNA protocol (with the exception of the 4°C incubation and the 37°C resuspension) should be performed at room temperature (20 - 25°C) as quickly as possible.
- Before the **first** use of the washing solution, add **7 ml** of absolute ethanol to it.
- To increase the yield of RNA from particularly difficult samples, add 7 ml of mercaptoethanol to 700 µl lysing solution immediately before its use.
- RNase inhibitors, such as RNasin or vanadyl-ribonucleoside complexes, should be used when processing substrates rich in ribonucleases.