



SAMPLE PREPARATION

Aurum™ Total RNA Mini Kit

RNA Suitable for All Downstream Applications

RNA Isolation From a Variety of Sources

Applications relying on total RNA require that it be intact and highly pure. Sample integrity is essential for researchers in fields such as molecular diagnostics, gene expression, and functional genomics. The Aurum total RNA mini kit meets the stringent requirements of these research applications, producing high-quality, DNA-free total RNA from a wide range of starting materials. High yields of total RNA can be obtained from up to 2×10^6 cultured cells, 2.4×10^9 bacterial (gram-positive or gram-negative) cells, or 3×10^7 yeast cells per column.

Animal tissue (up to 20–40 mg depending on tissue type) and plant tissue (up to 60 mg) can be used as well. Samples purified using the Aurum total RNA mini kit are ready for use in any downstream application.

Sample Purity and Integrity

Isolation of total RNA for gene expression studies requires special precautions. Depending on the application, the isolated RNA must be largely intact and free of contaminating genomic DNA to ensure confident data analysis and interpretation. The Aurum total RNA mini kit ensures successful sample preparation by including RNase-free reagents and plasticware as well as lyophilized DNase I for an on-column digest. The resulting RNA is suitable for even the most demanding applications.



Aurum RNA binding columns on Aurum vacuum manifold.

BIO-RAD

Streamlined Purification Technology

The speed and effectiveness of silica binding-based nucleic acid purification kits have made them a popular choice among life science researchers. The Aurum total RNA mini kit incorporates silica membrane technology in a mini column format for fast and convenient sample processing. Using safe and effective aqueous RNase-free reagents, the Aurum total RNA mini kit produces clean, intact RNA in minimal time.

The mini columns have luer ends that allow you to choose isolation by vacuum filtration in addition to centrifugation. If vacuum purification is preferred, the protocol may be carried out on the Aurum vacuum manifold with the column adaptor plate for parallel processing of up to 18 samples at a time. The luer fittings of the columns and column adaptor plate allow effective, streamlined sample preparation.

The simple protocol begins with collection and enzymatic treatment (if necessary) of cells, followed by cell lysis in Aurum total RNA lysis solution. After addition of alcohol, the samples are applied to the mini columns. Three quick washes, which include a DNase I digest, follow. Samples are ready for use after they are eluted with warmed elution solution into RNase-free micro test tubes included in the kit.

Integrated Products for Genomic Studies

Expression profiling applications are rapidly accelerating the pace of genetic research and giving a genome-wide perspective of gene function and variation. The Aurum total RNA mini kit complements many of our consumable and instrumentation lines for accurate RNA quantitation and analysis. With the iCycler iQ™ real-time PCR system, the Ultramark™ microplate reader, ReadyAgarose™ precast gels, the VersaDoc™ and Gel Doc™ imaging systems, and the new VersArray™ microarray platform, Bio-Rad offers an integrated solution to all genome-related questions.

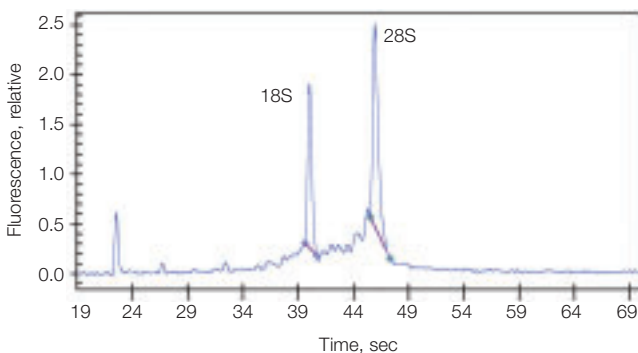


Fig. 1. Agilent 2100 bioanalyzer electropherogram of high-quality eukaryotic total RNA. Total RNA was isolated from 30 mg of rat brain using the Aurum total RNA mini kit in the spin format. Start times of the 18S and 28S ribosomal RNAs were 39.6 and 45.5 sec, respectively. The clearly defined peaks and minimal background fluorescence indicate intact, undegraded RNA.

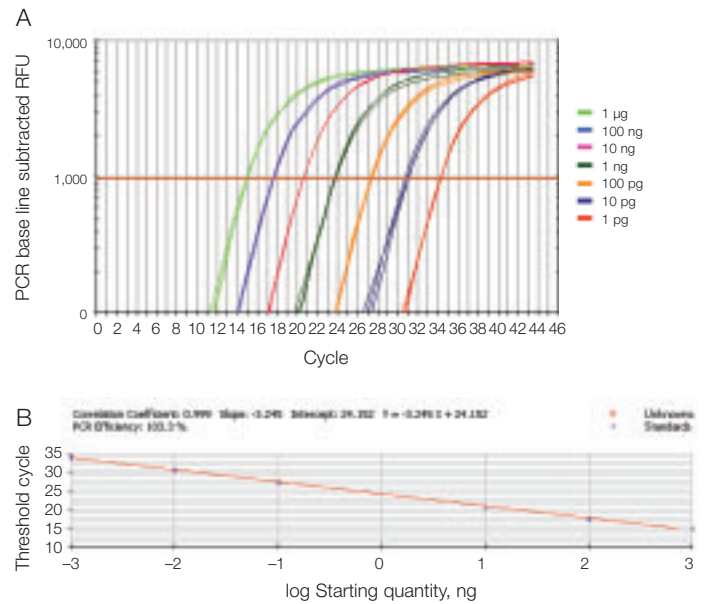


Fig. 2. Real-time RT-PCR with total RNA isolated using the Aurum total RNA mini kit. Total RNA isolated from a culture of 2×10^6 HeLa cells was eluted in Aurum RNA elution solution and a sample used in two-step real-time RT-PCR. Reverse transcription reactions were performed on 10-fold dilutions (1 µg to 1 pg) of the HeLa total RNA using the iScript™ cDNA synthesis kit. cDNA from these reactions was used to detect human β -actin gene expression levels using gene-specific primers and iQ™ SYBR® Green supermix. Real-time data were acquired on Bio-Rad's iCycler iQ system. A, real-time curves for detection of human β -actin cDNA; B, standard curve showing $r = 0.999$, efficiency = 103.3%.

Protocol Overview*

Aurum Total RNA Mini Kit — Vacuum Format

Animal tissue

Cut tissue into small pieces (<5 mm).
Grind into fine powder under liquid nitrogen.
Do not let tissue thaw.

Transfer up to 20 mg (hard tissue) or
40 mg (soft tissue) to a capped 2 ml tube.



Plant tissue

Cut tissue into small pieces (<5 mm).
Grind into fine powder under liquid nitrogen.
Do not let tissue thaw.

Transfer up to 60 mg to
a capped 2 ml tube.

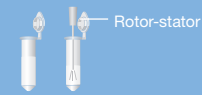


Continue with the following steps for all sample types:

Add 700 µl lysis solution.

Disrupt vigorously with rotor-stator for 30–60 sec.

700 µl lysis solution



Centrifuge lysate at maximum speed for 3 min.

Transfer supernatant to a new 2 ml capped tube.



Add 700 µl EtOH (use 60% for animal tissue, 70% for plant tissue) to supernatant.

Homogenize with rotor-stator 30 sec.

700 µl 60% EtOH or 70% EtOH



Assemble manifold properly for isolation.

Transfer lysate.

Apply vacuum.

Homogenized lysate



Add 700 µl low stringency wash.

Apply vacuum.

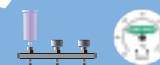
700 µl low stringency wash



Dilute 5 µl reconstituted* DNase I with 75 µl DNase dilution solution.

Add 80 µl diluted DNase I.
Incubate at room temperature 25 min for animal tissue, 15 min for plant tissue. Apply vacuum.

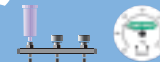
80 µl DNase I in dilution solution



Add 700 µl high stringency wash.

Apply vacuum.

700 µl high stringency wash



Add 700 µl low stringency wash.

Apply vacuum. Spin-purge 2 min into 2 ml capless tube.

700 µl low stringency wash



Place RNA binding column into a 1.5 ml capped tube.



Add 80 µl 70°C elution solution onto membrane stack.

Incubate 1 min. Centrifuge 2 min to elute.

80 µl elution solution



* Refer to manual for detailed protocol.

Features

- Parallel processing of samples
- Aqueous isolation procedure
- Fast, convenient protocol with silica binding
- Highly intact and pure total RNA for all downstream applications
- Sample types and amounts:
 - 2 x 10⁶ cultured cells
 - 2.4 x 10⁹ bacterial cells
 - 3 x 10⁷ yeast cells
 - ≤40 mg animal tissue
 - ≤60 mg plant tissue
- Lyophilized DNase I included
- RNase-free reagents and plasticware

Specifications

Format	Mini column
Method	Spin- or vacuum-mediated silica binding
Membrane binding capacity	>40 µg purified total RNA
Purity	A ₂₆₀ /A ₂₈₀ of 1.9–2.1
Stability	6 months for reagents

Ordering Information

Catalog #	Description
732-6820	Aurum Total RNA Mini Kit, 50 preps, includes 50 RNA binding columns, 50 capless collection tubes (2.0 ml), 2 x 50 capped sample tubes (2.0 ml), 50 capped sample tubes (1.5 ml), 1 vial lyophilized DNase I, RNase-free reagents, protocol overview, instructions
732-6800	Aurum Total RNA 96 Kit, 2 x 96-well preps, includes 2 grow blocks, 2 growth membranes, sealing tape, 2 RNA binding plates, 2 collection microplates, 2 vials lyophilized DNase I, RNase-free reagents, protocol overview, instructions
732-6470	Aurum Vacuum Manifold, includes column adaptor plate, 4 replacement luer caps, A stage and B stage, waste collection tray, vacuum regulator and gauge, tubing, protocol overview, instructions
170-8890	iScript cDNA Synthesis Kit, 25 x 20 µl reactions, includes 5x iScript reaction mix, iScript enzyme mixture, nuclease-free water
170-8891	iScript cDNA Synthesis Kit, 100 x 20 µl reactions, includes 5x iScript reaction mix, iScript enzyme mixture, nuclease-free water

Please ask your local Bio-Rad representative for a trial size of the Aurum total RNA kit.

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BIO-RAD

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Group

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