SAMPLE PREPARATION

Aurum™ Total RNA 96 Kit

High-Quality RNA in a High-Throughput Format

96-Well Total RNA Isolation

Pure, undegraded, full-length RNA is generally a critical requirement for all areas of RNA research. Most applications requiring total RNA demand high-quality samples. The Aurum total RNA 96 kit reproducibly isolates DNA-free total RNA from up to 1 x 10^6 cultured cells, 8 x 10^8 bacterial (gram-positive or gram-negative) cells, or 2 x 10^7 yeast cells per well in a 96-well plate format. The isolated RNA is of the highest integrity (Figure 1) and is suitable for the most discriminating applications (Figure 2).

High Sample Purity and Integrity

Gene expression applications such as quantitative real-time RT-PCR, northern hybridization, and microarray analysis sometimes cannot tolerate even the slightest DNA contamination or degradation of the RNA. However, these problems are inherent in RNA isolation processes due to the presence of endogenous genomic DNA and RNases. Cell harvesting, reagent preparation, and sample storage are particularly susceptible to factors that can affect the final quality of the isolated RNA. Accurate data analysis and interpretation require that contamination and degradation, whether due to endogenous or exogenous sources, be controlled throughout the purification process. To minimize these concerns, the Aurum total RNA 96 kit includes RNase-free reagents and plasticware as well as lyophilized DNase I for an in-well digest.

Aurum total RNA binding plate on Aurum vacuum manifold.

BIO-RAD

Safe, Rapid Purification Technology

Time-consuming and hazardous sample preparation procedures have given way to faster, safer protocols that produce RNA of equal or higher purity. Using silica membrane technology and optimized aqueous reagents, the Aurum total RNA 96 kit produces clean, reproducible total RNA for all major downstream applications. After initial sample collection and enzymatic digestion (if necessary), cells are disrupted and homogenized in the Aurum total RNA lysis buffer. The addition of alcohol, three wash steps that include a DNase digest, and an elution step follow. Purified RNA is recovered in high yield by saturating the silica membrane with warmed elution solution. Unlike many RNA purification kits, the Aurum total RNA 96 kit includes lyophilized DNase I. The entire purification process is completed in 60 minutes or less.

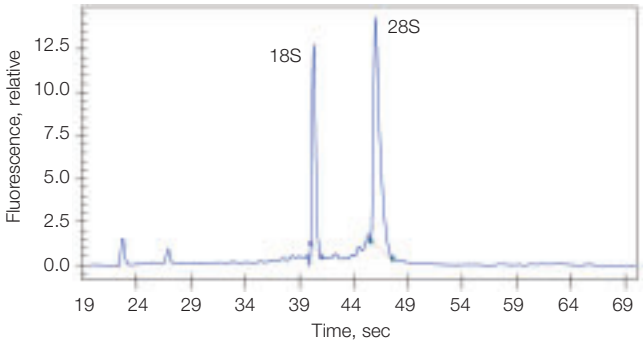


Fig. 1. Agilent 2100 bioanalyzer electropherogram of high-quality eukaryotic total RNA. Total RNA was isolated from a homogeneous culture of 1 x 10^6 NIH 3T3 cells using the Aurum total RNA 96 kit. Start times of the 18S and 28S ribosomal RNAs were 40.0 and 45.6 sec, respectively. The clearly defined peaks and minimal background fluorescence indicate intact, undegraded RNA.

Streamlined Preparation

Like all Aurum plates, the total RNA 96 binding plate is designed for use on the Aurum vacuum manifold — the only manifold of its kind on the market that is adaptable to both plates and columns. The two-stage vacuum manifold helps streamline the binding, washing, and elution steps of the purification process. Although the Aurum total RNA 96 protocol incorporates a DNase digest, it is entirely vacuum-mediated and does not require the extra centrifugation steps of other plate-based kits.

Integrated With Downstream Applications

New discoveries about RNA functions in cells are increasing the research focus on these molecules, making tools for accurate RNA quantitation and characterization a necessity. The Aurum total RNA 96 kit complements many consumable and instrumentation product lines for gene expression applications including the iCycler iQ™ system and iScript™ cDNA synthesis kits for quantitative real-time RT-PCR, ReadyAgarose™ precast high-throughput gels, the Ultramark™ microplate reader, the VersaDoc™ and Gel Doc™ imaging systems, and the new VersArray™ colony pickers, arrayers, and microarray readers.

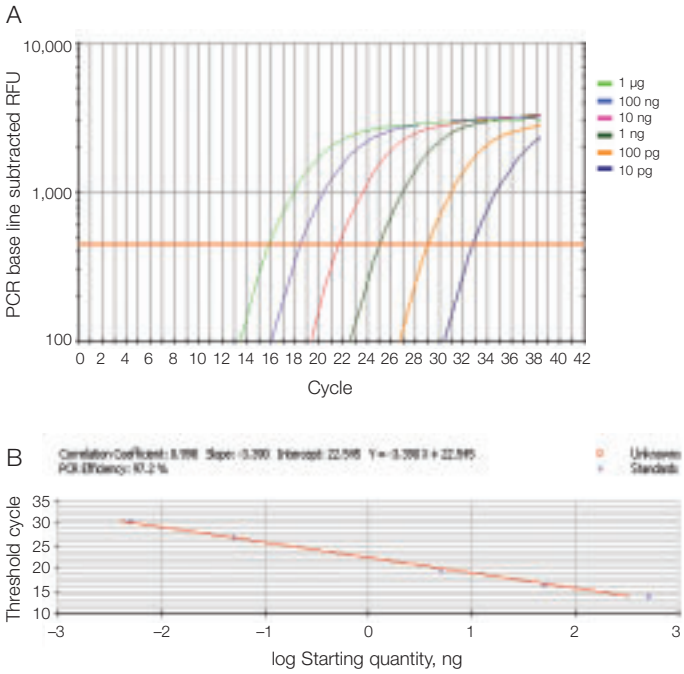


Fig. 2. Real-time RT-PCR with total RNA isolated using the Aurum total RNA 96 kit. Total RNA isolated from a culture of 1 x 10^6 HeLa cells was used in two-step real-time RT-PCR. Reverse transcription reactions were performed on 10-fold dilutions (1 µg to 10 pg) of the HeLa total RNA using the iScript cDNA synthesis kit. cDNA from these reactions was used to detect human α-tubulin 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 α-tubulin cDNA; B, standard curve showing r = 0.998, efficiency = 97.2%.

Protocol Overview*

Aurum Total RNA 96 Kit

Cultured cells

Adherent
Rinse vessel with PBS, aspirate.

Lyse in vessel if # of cells <1 x 10^6.

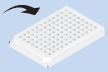
Nonadherent
Rinse with PBS.

Transfer up to 1 x 10^6 cells per microplate well.

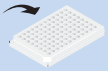
Centrifuge plate at 300 x g for 5 min.

Aspirate supernatant.

Add 150 µl lysis solution. Pipet up and down.



Add 150 µl 70% EtOH. Pipet up and down.



Bacterial cells

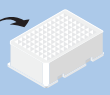
Transfer up to 8 x 10^8 cells into each well of a grow block.

Centrifuge at 1,500 x g for 10 min. Decant supernatant.

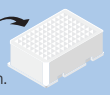
Add 100 µl of 500 µg/ml lysozyme. Pipet up and down.

Incubate at room temperature for 5 min.

Add 350 µl lysis solution. Pipet up and down.



Add 250 µl 70% isopropyl alcohol. Pipet up and down.



Yeast cells

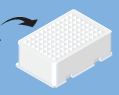
Transfer up to 2 x 10^7 cells into each well of a grow block.

Centrifuge at 1,500 x g for 10 min. Decant supernatant.

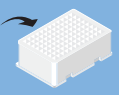
Add 1 ml of 50 U/ml lyticase in lyticase dilution buffer. Pipet up and down.

Incubate at room temperature for 10 min. Centrifuge at 1,500 x g for 5 min. Discard supernatant.

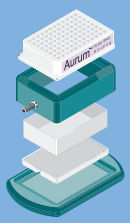
Add 350 µl lysis solution. Pipet up and down.



Add 350 µl 70% EtOH. Pipet up and down.



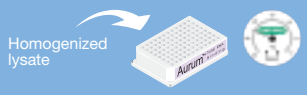
Continue with the following steps for all sample types:



Assemble manifold properly for isolation.

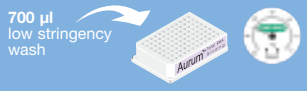
Transfer lysate to wells of RNA binding plate.
Apply vacuum.

Homogenized lysate



Add 700 µl low stringency wash to each well.
Apply vacuum.

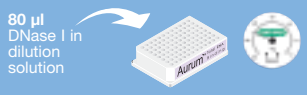
700 µl low stringency wash



For each well, dilute 2.5 µl reconstituted* DNase I with 77.5 µl DNase dilution solution.

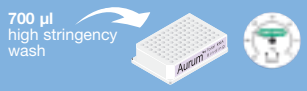
Add 80 µl of diluted DNase I to each well.
Incubate 10 min at room temp. Apply vacuum.

80 µl DNase I in dilution solution



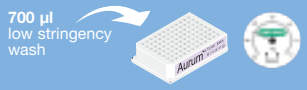
Add 700 µl high stringency wash to each well.
Apply vacuum.

700 µl high stringency wash



Add 700 µl low stringency wash to each well.
Apply vacuum.

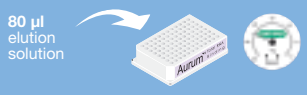
700 µl low stringency wash



Assemble manifold properly for elution.

Add 80 µl 70°C elution solution per well onto membrane stack.
Incubate 1 min. Apply vacuum gradually to –20 to –23" Hg. Continue to apply vacuum for 5 min to elute.

80 µl elution solution



* Refer to manual for detailed protocol.

Features

- High-throughput total RNA isolation
- Aqueous isolation procedure
- Quick protocol utilizing silica binding
- High-quality total RNA for various applications
- Sample types and amounts:
  1 x 10^6 cultured cells
  8 x 10^8 bacterial cells
  2 x 10^7 yeast cells
- Lyophilized DNase I included
- RNase-free reagents and plasticware

Specifications

Format	96-well plate, manual and automated*
Method	Vacuum-mediated silica binding
Membrane binding capacity	≥40 µg purified total RNA (per well)
Purity	A_{260}/A_{280} of 1.9–2.1
Stability	6 months for reagents

* The Aurum total RNA 96 kit has been optimized for use on the PerkinElmer MultiPROBE II HT EX automated liquid handling system with gripper integration platform. Please inquire for protocol.

Ordering Information

Catalog #	Description
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
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
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.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

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