PureZOL™ RNA Isolation Reagent

Instruction Manual

Catalog #732-6890

For technical support,
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Table of Contents

Section 1
Introduction

PureZOL RNA isolation reagent is intended for the extraction of total RNA from animal and plant tissues, cultured mammalian cells, and bacterial and yeast cells in under 1 hour. PureZOL can also be used for the simultaneous extraction of RNA, DNA, and proteins from various samples. This reagent allows processing of small amounts of starting material (50 cells or 5 mg of tissue), and can be scaled up to process large amounts of starting material.

PureZOL RNA isolation reagent is a monophasic solution of phenol and guanidine isothiocynate, an improved version of the single-step isolation of total RNA developed by Chomczynski and Sacchi (1987). The reagent facilitates immediate and effective inhibition of RNase activity, while lysing cells and eliminating other cellular components. Following the addition of chloroform and subsequent centrifugation, the homogenate separates into an aqueous phase, an interphase and an organic phase. RNA is recovered in the aqueous phase with the addition of isopropyl alcohol, and is subsequently washed in ethanol and solubilized in RNase-free water.

Total RNA isolated from this procedure is generally DNA- and protein-free and can be used for northern blot analysis, *in vitro* translation, poly $(A)^+$ selection, RNase protection assays, RT-PCR, and molecular cloning. For RT-PCR analysis, DNase treatment may be necessary for optimal results. Subsequent cleanup of RNA using an Aurum™ total RNA mini kit (732-6820) is recommended to remove any phenol and other contaminants that may have coprecipitated with the RNA (see section 8 for ordering information).

Kit Components

Catalog # 732-6890 – PureZOL RNA isolation reagent, 100 ml

Storage and Stability

PureZOL RNA isolation reagent is shipped at room temperature. This product is guaranteed for 12 months from the date of purchase when stored at 2–8°C and kept away from light.

Special Handling Instructions

PureZOL RNA isolation reagent contains a poison (phenol) and an irritant (guanidine thiocynate). The reagent causes burns and can be fatal if ingested. When working with PureZOL, use gloves and eye protection (laboratory glasses, shield, and safety goggles). Do not get on skin or clothing. Avoid breathing vapor. Read warning notice on bottle and MSDS.

In case of contact: *Immediately flush eyes or skin with a large amount of water for at least 15 minutes and seek immediate medical attention.*

Section 2
Materials and Equipment Required (Not Provided)

Microcentrifuges (capable of >12,000 x g) — at 4°C and at room temperature

95–100% ethanol, ACS grade or better

Chloroform (without additives such as isoamyl alcohol); need 0.2 ml of chloroform per 1 ml of PureZOL

Isopropyl alcohol; need 0.5 ml per 1 ml of PureZOL

RNase-free water (DEPC-treated water)

75% ethanol (prepared in DEPC-treated water); need 1 ml per 1 ml of PureZOL

RNase-free pipet tips and micropipets

RNase-free polypropylene centrifuge tubes with caps, capable of withstanding centrifugal forces of 12,000 x g

Disposable latex or vinyl gloves

Supplies for tissue grinding, disruption, and homogenization

- Fresh tissue — tissue cutter and a ruler to measure size
- Frozen tissue — liquid nitrogen and mortar and pestle
- Tissue homogenizer — dounce homogenizers, rotor-stator homogenizers, or bead mill homogenizers are recommended

Optional — Aurum total RNA mini kit (732-6820) for RNA cleanup following isolation of RNA using PureZOL (see section 8 for ordering information)

Section 3
Maintaining an RNase-Free Environment

To avoid introducing RNases, great care must be taken in handling the reagents and purified RNA samples. Care must be taken to proceed through the RNA isolation as quickly as possible. An RNase-free environment will yield the best results.

If possible, work in an RNase-free environment; use latex or vinyl gloves when handling reagents or RNA and change gloves frequently.

Nondisposable, nonautoclavable plasticware should be rinsed with 0.1 M NaOH, 1 mM EDTA followed by several rinses with diethyl pyrocarbonate (DEPC)-treated water before use. DEPC is an efficient, strong, and nonspecific RNase inhibitor that is usually used at a concentration of 0.1%. ***Warning***: *DEPC is suspected to be a carcinogen and should be handled with care. Always use gloves and open under a fume hood.*

Glassware and other autoclavable items may be treated using the DEPC method described above for nonautoclavable plasticware, or by baking for 4 hours at 300°C.

Working surfaces and pipets should be kept clean and wiped periodically with a 0.5 M NaOH solution.

Use sterile plasticware and machine-packaged aerosol-resistant pipet tips. Pour tubes from an unopened bag (or bag marked "For RNA Use Only") onto an RNase-free environment (such as plastic wrap). Otherwise, sterilize by autoclaving.

Solutions that are prepared by the user should be treated with DEPC to inactivate RNases. See instructions below on how to prepare DEPC-treated solutions.

Keep reagent bottles closed when not in use and keep RNA samples on ice to prevent degradation by RNases.

Preparing Reagents Not Supplied With the Kit

Note*: DEPC is destroyed by primary amines. If a solution containing a primary amine will be DEPC-treated, omit the amine in preparing the solution. Perform the DEPC treatment as described above, and add the amine to the autoclaved solution once the solution has cooled.*

Preparation of DEPC-treated water. To prepare a 0.1% (v/v) solution of DEPC-treated water, add 1.0 ml of liquid DEPC per 1 L of water. Incubate the solution at 37°C for 1 hour while mixing thoroughly. Autoclave the treated water to remove the DEPC.

Preparation of 75% ethanol. To prepare 100 ml of 75% ethanol, add 75 ml of 95–100% ethanol to 25 ml of DEPC-treated water. Mix well before use.

Section 4
Recommendations for Best Results

- When isolating RNA from small sample sizes (<500,000 cells or <10 mg of tissue), lyse or homogenize in 0.8 ml of PureZOL. Use glycogen as an RNA carrier by adding 5 µl of a 20 mg/ml glycogen solution (not provided) to the aqueous phase before precipitation with isopropyl alcohol. Carry out precipitation for 30 minutes at 4°C.
- Frozen tissues should be kept at –70°C prior to homogenization and thawed in the appropriate volume of PureZOL RNA isolation reagent.
- An additional step may need to be performed for samples with a high content of proteins, lipid, polysaccharides, or extracellular material, such as muscles, fat tissue, and tuberous parts of plants. Following homogenization, remove insoluble material by centrifuging the homogenate at 12,000 x g for 10 minutes at 4°C. The resulting pellet contains the insoluble, extracellular components, such as polysaccharides and high molecular weight material, while the supernatant contains the RNA. In samples from fat tissue, the excess layer of fat that collects at the top should be removed. Transfer the cleared homogenate to a new RNase-free tube and continue with the RNA extraction protocol.

Section 5
Protocol

Carry out all steps at room temperature unless otherwise indicated. RNase-free disposable polypropylene tubes should be used throughout the procedure. The entire procedure should take less than 1 hour.

1. **Disrupt and homogenize the sample using the following suggestions depending on the sample type:**

   Cells Grown in a Monolayer

   Cells grown in a monolayer should be lysed with PureZOL directly in the culture dish. Aspirate the culture medium and immediately add 1 ml of PureZOL to a 10 cm^2 dish. Pipet up and down several times. The amount of PureZOL added is dependent on the area of culture dish (1 ml per 10 cm^2) and not on cell number. Insufficient volumes of PureZOL may result in DNA contamination. **Note**: Do not wash cells prior to the addition of PureZOL as this could increase the possibility of mRNA degradation.

   Suspension Cells (Mammalian, Plant, Bacterial, or Yeast)

   Pellet the cells by centrifuging at 3,000–5,000 x g for 2 minutes. Immediately lyse by adding 1 ml of PureZOL to 1 x 10^7 cultured mammalian and plant cells, 2.4 x 10^9 of Gram-positive or Gram-negative bacteria, or 3.0 x 10^7 of yeast in a suitable sized tube. Pass the lysate through a pipet or an 18-gauge needle and syringe several times. To improve the efficiency of the cell lysis process, a rotor-stator or bead mill homogenizer is

recommended to disrupt the cell walls of yeast and bacteria. Bacteria and yeast lysate can also be heated to 55°C for 10 minutes prior to adding chloroform to increase the effectiveness of lysis by PureZOL. **Note**: Do not wash cells prior to the addition of PureZOL as this could increase the possibility of mRNA degradation.

Fresh Tissue

Freshly harvested tissue samples should be processed immediately after dissection to avoid RNA degradation. Alternatively, the tissue can be immediately frozen in liquid nitrogen and processed using instructions for frozen tissue.

To process a freshly dissected tissue, add 1 ml of PureZOL for every 50–100 mg of tissue in a suitable sized tube for disruption and homogenization, and homogenize the sample for 30–60 seconds using a rotor-stator or bead mill homogenizer (refer to manufacturer's instructions for details). Although not as effective, passing the tissue sample through an 18-gauge needle and syringe can be used for sample disruption if a homogenizer is not available. Pass the sample through the needle and syringe until no more solid tissue is left in the lysate. The sample volume should not exceed 10% of the volume of PureZOL used for disruption.

Frozen Tissue

Grind the frozen tissues to a fine powder with a mortar and pestle under liquid nitrogen. Avoid thawing the sample, by periodically adding liquid nitrogen to the mortar. Weigh up to

100 mg of tissue and transfer the sample into a suitable sized tube for disruption and homogenization. Add 1 ml of PureZOL reagent to every 50–100 mg of the frozen ground tissue and immediately homogenize for 30–60 seconds using a rotor-stator or bead mill homogenizer (refer to manufacturer's instructions for details). Alternatively, take a small chunk of the frozen tissue (equivalent to 50–100 mg) and drop it into 1 ml of PureZOL reagent and immediately homogenize the sample. The sample volume should not exceed 10% of the volume of PureZOL used for homogenization.

2. **Incubate the lysate at room temperature for 5 minutes once the sample has been disrupted in PureZOL, to allow the complete dissociation of nucleoprotein complexes.**

   Following the disruption step, the sample can be stored at –70°C for at least 1 month. To process frozen lysates, samples should be thawed at room temperature. If necessary, heat samples to 37°C in a water bath for 5–10 minutes to completely dissolve salts. Avoid extended treatment at 37°C since this can cause chemical degradation of the RNA.

   Note: It is recommended that lysate from tissues that are rich in fat, polysaccharides, proteins, and extracellular material be centrifuged at 12,000 x g for 10 minutes at 4°C following the 5 minute incubation at room temperature. This step removes any solid insoluble debris that was left after the disruption step. Transfer the supernatant into a new 2.0 ml microcentrifuge tube without aspirating the pellet, then proceed to step 3. For lipid-rich samples, avoid transferring the excess fat that collects as a

top layer. Carryover of the solid debris can cause column clogging and affect RNA sample purity.

3. **Add 0.2 ml of chloroform per 1 ml of PureZOL used in step 1, then cover and shake vigorously for 15 seconds. Do not vortex.**

4. **Incubate for 5 minutes at room temperature while periodically mixing the sample.**

5. **Centrifuge at 12,000 x g for 15 minutes at 4°C.**

   Following centrifugation, the mixture will separate into 3 phases: an upper, colorless aqueous phase, a white interphase, and a lower, red organic phase. RNA will be exclusively in the aqueous phase, while DNA and proteins remain in the interphase and organic phase. The volume of the aqueous phase should be approximately or 60% of the volume of PureZOL used in the initial disruption.

6. **Without disturbing the interphase, immediately transfer the aqueous phase to a new RNase-free tube.**

   Note: It is crucial that none of the interphase or organic phase be transferred with the aqueous phase. Some of the aqueous phase should be left behind to avoid the risk of contaminating the RNA with contaminants such as phenol, which can interfere with downstream applications.

7. **Add 0.5 ml of isopropyl alcohol per 1 ml of PureZOL used in step 1. Mix thoroughly and then incubate at room temperature for 5 minutes.**

8. **Centrifuge at 12,000 x g for 10 minutes at 4°C.**

9. **The RNA will appear as a white pellet on the side and bottom of the tube. Carefully discard the supernatant.**

10. **To wash the RNA pellet, add 1 ml of 75% ethanol for every 1 ml of PureZOL used in step 1**.

    At this point, the sample can be stored in ethanol at 4°C for at least 1 week or at -20°C for at least 1 year.

11. **Vortex the sample and then centrifuge at 7,500 x g (max) for 5 minutes at 4°C. Carefully discard the supernatant**.

12. **Air-dry the RNA pellet for about 5 minutes**. Do not let the RNA pellet dry completely since this will decrease solubility. Note: Do not use centrifugation by vacuum (Speed Vac).

13. **Resuspend the pellet in the appropriate volume of RNase-free water (DEPC-treated water)**. Pipet up and down a few times to completely resuspend the pellet. It may be necessary to incubate at 55–60°C for 10 minutes to completely dissolve the RNA pellet.

    Note: We recommend subsequent cleanup of the RNA using an Aurum total RNA mini kit (732-6820) to remove any phenol or other contaminants that may have coprecipitated with the RNA. See section 8 for ordering information.

14. **The extracted RNA can be used immediately in downstream applications**. Alternatively, the RNA sample can be aliquoted and stored at –20°C for 1 month or at –70°C for 1 year. Avoid freeze-thaw cycles.

Section 6
Troubleshooting

Problem	Possible Cause	Recommended Solution
Incomplete separation of phases after centrifugation	Lysate was not mixed properly after adding chloroform (See step 3 in protocol)	Once chloroform is added, mix tubes vigorously by shaking for 15 seconds. Do not vortex. Let the lysate incubate for 5 minutes at room temperature, then mix again before centrifuging
	Lysate was not centrifuged at the right temperature	Make sure that the centrifugation is performed at 4°C following the addition of chloroform in order to achieve complete separation of the phases
	Incorrect amount of chloroform was added	For every 1 ml of PureZOL used, add 0.2 ml of chloroform
Low yield	Incomplete disruption and homogenization of sample	Increase the duration or intensity of sample disrup tion.Scale up the amount of PureZOL used based on sample size
	Over dried RNA pellet	Do not use a Speed Vac to dry the pellet. Dry pellet briefly at room temperature for 5 minutes. Add DEPC-treated water to the tube and pipet up and down several times to resuspend

Problem	Possible Cause	Recommended Solution
Low yield (continued)		the pellet. If necessary, incubate the resuspended pellet for 10 minutes at 55–60°C and then pipet up and down
	Low amount of starting material	When isolating RNA from small sample amounts, homogenize the sample in 0.8 ml of PureZOL. Use glycogen as an RNA carrier by adding 5 µl of a 20 mg/ml glycogen solution (not provided) to the aqueous phase before precipitation with isopropyl alcohol. Carry out precipitation for 30 minutes at 4°C
	The solution used to dilute the RNA for spectrophotometric reading has a low	A_{260}/A_{280} may vary based on the pH of the solution used todilute RNA samples. To get more accurate and consistent A_{260} and A_{280} values, dilute your RNA samples with a solution that has a pH within the range 6.5 to 8.5

Problem	Possible Cause	Recommended Solution
Low yield (continued)	Insufficient amount of PureZOL used for sample lysis and homogenization	Scale up the amount of PureZOL used according to sample size. Sample volume should not exceed 10% of the PureZOL reagent used for homogenization
	Lysate was not incubated at room temperature for 5 minutes after the disruption step (see step 4 in protocol)	Make sure to incubate the lysate after the disruption step for 5 minutes at room temperature to allow complete dissociation of nucleoprotein complexes
RNA is degraded	RNase contamination of solutions supplied by the user	Treat all user-made solutions with DEPC before use (see section 3, Maintaining an RNase-Free Environment)
	RNase contamination of plasticware and work station	See section 3, Maintaining an RNase-Free Environment
	Frozen tissue samples were allowed to thaw or sit at room temperature	Add PureZOL directly to frozen samples before they thaw. Do not let starting samples sit at room temperature

Problem	Possible Cause	Recommended Solution
RNA is degraded (continued)	Cells grown in either monolayer or suspension were washed prior to homogenization with PureZOL	For cells grown in monolayer, aspirate the growth medium and then add PureZOL directly to the plate. No washing or trypsinization is necessary. For cells grown in suspension, pellet the cells and aspirate growth medium, then add PureZOL directly to the pellet
	Starting tissue sample was not immediately frozen, or had gone through several freeze-thaw cycles before RNA purification was performed	Make sure that starting material is immediately processed following dissection. Alternatively, the starting material can be immediately frozen after dissection. Once frozen, do not subject starting material to freeze-thaw cycles.
	Cultured cells were dispersed by trypsin	Cells should be lysed directly in PureZOL RNA isolation reagent. Do not wash cells or trypsinize prior to lysing
Genomic DNA contamination	Some of the white interphase (after phase separation) was transferred with the aqueous phase	Leave some of the aqueous phase solution behind to avoid transferring the white interphase with the aqueous phase (see step 6 in the protocol)

Problem	Possible Cause	Recommended Solution
Genomic DNA contamination (continued)	Phase separation not performed at the right temperature	Make sure that centrifugation step is performed at 4°C following the addition of chloroform in order to achieve complete separation of the phases
	Insufficient amount of PureZOL used for sample lysis and homogenization	Scale up the amount of PureZOL used according to sample size. Sample volume should not exceed 10% of the PureZOL reagent used for homogenization.
RNA contamination with extracellular material	Starting material is high in fat, proteins, or polysaccharides	After the sample disruption step, centrifuge the lysate at 12,000 x g for 10 minutes at 4°C to pellet any debris. Transfer the lysate into a new RNase-free tube, leaving behind the pellet. This should be done before adding the chloroform.

Section 7 Reference

Chomczynski P and Sacchi N, Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction, Anal Biochem 162, 156–159 (1987)

Section 8
Ordering Information

Catalog #	Description
732-6890	PureZOL RNA isolation reagent, 100 ml

Related Products

Aurum Total RNA Kits for Isolation of High Quality RNA From a Variety of Samples

732-6820	Aurum Total RNA Mini Kit, 50 preps, includes 50 RNA binding columns, 50 capless collection tubes (2.0 ml), 100 capped microcentrifuge tubes (2.0 ml), 50 capped microcentrifuge tubes (1.5 ml), reagents, protocol overview, instructions
732-6800	Aurum Total RNA 96 Kit, 2 x 96 well preps, includes 2 grow blocks, 2 growth membranes, 2 RNA binding plates, 2 micro collection plates, reagents, protocol overview, instructions

Aurum Total RNA Kits for Isolation of High Quality RNA From Difficult-to-Process Samples

732-6830	Aurum Total RNA Fatty and Fibrous Tissue Kit, 50 preps, includes 50 ml PureZOL RNA isolation reagent, 50 RNA binding mini columns, 50 capless collection tubes (2.0 ml), 100 capped sample tubes (2.0 ml), 50 capped sample tubes (1.5 ml), 1 vial lyophilized DNase I, RNase-free reagents and plasticware, protocol overview, instructions

Legal Notices

PureZOL RNA isolation reagent is subject to US Patent 5,346,994.
Speed Vac is a trademark of Sevant Instruments, Inc.

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