YTzol Pure RNA

Contents and storage

Contents	Cat No: YT9066	Cat No: YT9065	Cat No: YT9064
YTzol pure RNA	20 mL	50 mL	100 mL

Product information

YTzol Pure RNA solution is a ready-to-use reagent, considered to extract high quality total RNA (plus DNA and proteins) from cell and tissue samples of human, animal, plant, yeast, or bacterial origin, during one hour. YTzol Pure RNA solution is a monophasic solution of phenol , guanidine isothiocyanate , and other exclusive mechanisms which simplify the isolation of a range of RNA types of large or small molecular size. YTzol Pure RNA solution keeps the integrity of the RNA due to highly active inhibition of RNase activity while disrupting cells and dissolving cell components during sample homogenization.

YTzol Pure RNA solution permits for concurrent processing of a large number of samples . YTzol Pure RNA solution allows to complete sequential precipitation of RNA, DNA, and proteins from a single sample .

After homogenizing the sample with YTzol Pure RNA solution, chloroform is added, and the homogenate is allowed to separate into a clear upper aqueous layer (containing RNA), an interphase, and a red lower organic layer

(containing the DNA and proteins). RNA is precipitated from the aqueous layer with isopropanol. DNA is precipitated from the interphase/organic layer with ethanol. Protein is precipitated from the phenol-ethanol supernatant by isopropanol precipitation. The precipitated RNA, DNA, or protein is washed to remove impurities, and then resuspended for use in downstream applications.

• Isolated RNA can be used in RT-PCR, Northern Blot analysis, Dot Blot hybridization, poly(A)+ selection, in vitro translation, RNase protection assay, and molecular cloning.

• Isolated DNA can be used in PCR, Restriction Enzyme digestion, and Southern Blots.

• Isolated protein can be used for Western Blots, recovery of some enzymatic activity, and some immunoprecipitation.

YTzol Pure RNA solution can also be used with Phasemaker™ Tubes to isolate RNA.

Required materials not supplied

Table 1 :
Materials required for RNA, DNA, and protein isolation

Item
Equipment
Centrifuge and rotor capable of reaching 12,000 × g and 4°C
Tubes
Polypropylene microcentrifuge tubes
Reagents
Chloroform

Table 2 :
Materials required for RNA isolation

Item
Equipment
Water bath or heat block at 55–60°C
Reagents
Isopropanol
Ethanol, 75%
RNase-free water of 0.5% SDS
(Optional) RNase-free glycogen

Table 3:
Materials required for DNA isolation

Item
Reagents
Ethanol, 100%
Ethanol, 75%
0.1 M sodium citrate in 10% ethanol
8 mM NaOH
HEPES

Table 4:
Materials required for protein isolation

Item
Equipment
(Optional) Dialysis membranes
Reagents
Isopropanol
Ethanol, 100%
0.3 M Guanidine hydrochloride in 95% ethanol
1% SDS

Input sample requirements

Note : Perform RNA isolation immediately after sample collection or quick-freeze samples immediately after collection and store at –80°C or in liquid nitrogen until RNA isolation.

Sample type	**Starting material per 1 mL of** YTzol Pure RNA solution
Tissues[1]	50–100 mg of tissue
Cells grown in monolayer	1×10^5–1×10^7 cells grown in monolayer in a 3.5–cm culture dish (10 cm2)
Cells grown in suspension	5–10×10^6 cells from animal, plant, or yeasty origin or 1×10^7 cells of bacterial origin

[1] Fresh tissues or tissues stored in RNA Later Solution (Cat. No. YT9085).

Procedural guidelines

• Perform all steps at room temperature (20–25°C) unless otherwise noted.
• Use cold YTzol Pure RNA solution if the starting material contains high levels of RNase, such as spleen or pancreas samples.
• Use one-use, individually wrapped, sterile plastic ware and sterile, disposable RNase-free pipettes, pipette tips, and tubes.
• Wear disposable gloves while handling reagents and RNA samples to avoid RNase contamination from the surface of the skin; change gloves regularly, mostly as the protocol progresses from basic extracts to more purified materials.
• Always use proper microbiological sterilized techniques when working with RNA.

Lyse samples and separate phases

1. Lyse and homogenize samples in YTzol Pure RNA solution according to your starting material.

• **Tissues:**
Add 1 mL of YTzol Pure RNA solution per 50–100 mg of tissue to the sample and homogenize using a homogenizer.

• **Cell grown in monolayer:**
a. Remove growth media.
b. Add 0.3–0.4 mL of YTzol Pure RNA solution per 1×10^5—10^7 cells directly to the culture dish to lyse the cells.
c. Pipet the lysate up and down several times to homogenize.

• **Cells grown in suspension:**
a. Pellet the cells by centrifugation and discard the supernatant.
b. Add 0.75 mL of YTzol Pure RNA solution per 0.25 mL of sample (5–10×10^6 cells from animal, plant, or yeasty origin or 1 ×107 cells of bacterial origin) to the pellet.
Note: Do not wash cells before addition of YTzol Pure RNA solution to avoid mRNA degradation.
c. Pipet the lysate up and down several times to homogenize.
Note: The sample volume should not exceed 10% of the volume of YTzol Pure RNA solution used for lysis.

STOPPING POINT Samples can be stored at 4°C overnight or at –20°C for up to a year.

2. *(Optional)* If samples have a high fat content, centrifuge the lysate for 5 minutes at 12,000 × *g* at 4–10°C, then transfer the clear supernatant to a new tube.
3. Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.
4. Add 0.2 mL of chloroform per 1 mL of YTzol Pure RNA solution used for lysis, then securely cap the tube.
5. Incubate for 2–3 minutes.
6. Centrifuge the sample for 15 minutes at 12,000 × *g* at 4°C.
The mixture separates into a lower red phenol-chloroform, and interphase, and a colorless upper aqueous phase.
7. Transfer the aqueous phase containing the RNA to a new tube.
8. Transfer the aqueous phase containing the RNA to a new tube by angling the tube at 45° and pipetting the solution out.

IMPORTANT! Avoid transferring any of the interphase or organic layer into the pipette when removing the aqueous phase.

Proceed directly to "Isolate RNA" on page 2.
Save the interphase and organic phase if you want to isolate DNA or protein. See "Isolate DNA" on page 3 or "Isolate proteins" on page 4 for detailed procedures.
The organic phase can be stored at 4°C overnight.

Isolate RNA

1) Precipitate the RNA

a. *(Optional)* If the starting sample is small (<106 cells or <10 mg of tissue), add 5–10 µg of RNase-free glycogen as a carrier to the aqueous phase.
Note: The glycogen is co-precipitated with the RNA, but does not inhibit with following applications.
b. Add 0.5 mL of isopropanol to the aqueous phase, per 1 mL of YTzol Pure RNA solution used for lysis.
c. Incubate for 10 minutes.
d. Centrifuge for 10 minutes at 12,000 × *g* at 4°C.
Total RNA precipitate forms a white gel-like pellet at the bottom of the tube.
e. Discard the supernatant with a micropipettor.

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2) Wash the RNA

a. Resuspend the pellet in 1 mL of 75% ethanol per 1 mL of YTzol Pure RNA solution used for lysis.
Note: The RNA can be stored in 75% ethanol for at least 1 year at –20°C, or at least 1 week at 4°C.
b. Vortex the sample briefly, then centrifuge for 5 minutes at 7500 × *g* at 4°C.
c. Discard the supernatant with a micropipettor.
d. Vacuum or air dry the RNA pellet for 5–10 minutes.
Note : Do not dry the pellet by vacuum centrifuge. Do not let the RNA pellet dry, to ensure total solubilization of the RNA. Partially dissolved RNA samples have an $A_{230/280}$ ratio <1.6.

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3) Solubilize the RNA
Resuspend the pellet in 20–50 µL of RNase-free water, 0.1 mM EDTA, or 0.5% SDS solution by pipetting up and down.
Note : Do not dissolve the RNA in 0.5% SDS if the RNA is to be used in subsequent enzymatic reactions.
b. Incubate in a water bath or heat block set at 55–60°C for 10–15 minutes.
Proceed to downstream applications, or store the RNA at –70°C.

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4) Determine the RNA yield
Determine the RNA yield using one of the following methods.

Method	Procedure
Absorbance Absorbance at 260 nm provides total nucleic acid content, while absorbance at 280 nm determines sample purity. Since free nucleotides, RNA, ssDNA, and dsDNS absorb at 260 nm, they all contribute to the total absorbance of the sample.	1. Dilute sample in RNase-free water, then measure absorbance at 260 nm and 280 nm. 2. Calculate the RNA concentration using the formula A260 ×dilution × 40 = µg RNA/mL. 3. Calculate the A260/A280 ratio. A ratio of ~2 is considered pure. RNA samples can be quantified by absorbance without prior dilution using the NanoDrop™ Spectophotometer. Refer to the instrument's instructions for more information.

Table 5: Typical RNA ($A_{260/280}$ of >1.8) yields from various starting materials

Starting material	Quantity	RNA yield
Epithelial cells	1×10^6 cells	8–15 µg
New tobacco leaf	—	73 µg
Fibroblasts	1×10^6 cells	5–7 µg
Skeletal muscles and brain	1 mg	1–1.5 µg
Placenta	1 mg	1–4 µg
Liver	1 mg	6–10 µg
Kidney	1 mg	3–4 µg

Isolate DNA

Isolate DNA from the interphase and the lower phenol-chloroform phase saved from “Lyse samples and separate phases“ on page 2.

1) Precipitate the DNA
a. Remove any remaining aqueous phase covering the interphase.
This is critical for the quality of the isolated DNA.
b. Add 0.3 mL of 100% ethanol per 1 mL of YTzol Pure RNA solution used for lysis.
c. Cap the tube, mix by inverting the tube several times.
d. Incubate for 2–3 minutes.
e. Centrifuge for 5 minutes at 2000 × *g* at 4°C to pellet the DNA.
f. Transfer the phenol-ethanol supernatant to a new tube.
The supernatant is used for protein isolation (see “Isolate proteins“ on page 5, if needed, and can be stored at –70°C for several months.

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2) Wash the DNA
a. Resuspend the pellet in 1 mL of 0.1 M sodium citrate in 10% ethanol, pH 8.5, per 1 mL of YTzol Pure RNA solution used for lysis.
b. Incubate for 30 minutes, mixing infrequently by gentle inversion.
Note: The DNA can be stored in sodium citrate/ethanol for at least 2 hours.

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c. Centrifuge for 5 minutes at 2000 × *g* at 4°C.
d. Discard the supernatant with a micropipettor.
e. Repeat step 2a–step 2d once.
Note: Repeat step 2a–step 2d twice for large DNA pellets (>200 µg).
f. Resuspend the pellet in 1.5–2 mL of 75% ethanol per 1 mL of YTzol Pure RNA solution used for lysis.
g. Incubate for 10–20 minutes, mixing infrequently by gentle inversion.
Note: The DNA can be stored in 75% ethanol at several months at 4°C.
h. Centrifuge for 5 minutes at 2000 × *g* at 4°C.
i. Discard the supernatant with a micropipettor.
j. Vacuum or air dry the DNA pellet for 5–10 minutes.
Note : Do not dry the pellet by vacuum centrifuge.

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3) Solubilize the DNA
a. Resuspend the pellet in 0.3–0.6 mL of 8 mM NaOH by pipetting up and down.
Note: We recommend resuspending the DNA is a mild base because isolated DNA does not resuspend well in water or Tris buffer.
b. Centrifuge for 10 minutes at 12,000 × *g* at 4°C to remove insoluble materials.
c. Transfer the supernatant to a new tube, then adjust pH as needed with HEPES. Proceed to downstream applications, or store the DNA at 4°C overnight. For longer-term storage at –20°C , adjust the pH to 7–8 with HEPES and add 1 mM EDTA.

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4) Determine the DNA

Determine the DNA yield using one of the following methods.

	Procedure
Absorbance Absorbance at 260 nm provides total nucleic acid content, while absorbance at 280 nm determines sample purity. Since free nucleotides, RNA, ssDNA, and dsDNS absorb at 260 nm, they all contribute to the total absorbance of the sample.	1. Dilute sample in water or buffer (pH >7.5), then measure absorbance at 260 nm and 280 nm. 2. Calculate the DNA concentration using the formula A260 ×dilution × 50 = µg DNA/mL. 3. Calculate the A260/A280 ratio. A ratio of ~1.8 is considered pure. DNA samples can be quantified by absorbance without prior dilution using the NanoDrop™ Spectophotometer. Refer to the instrument's instructions for more information.

Table 6 : Typical DNA ($A_{260/280}$ of 1.6–1.8) yields from various starting materials

Starting material	Quantity	DNA yield
Fibroblasts	1×10^6 cells	5–7 µg
Cultured cells, mammal	1×10^6 cells	5–7 µg
Skeletal muscles and brain	1 mg	2–3 µg
Placenta	1 mg	2–3 µg
Liver	1 mg	3–4 µg
Kidney	1 mg	3–4 µg

Isolate proteins

Isolate the proteins from the phenol-ethanol supernatant saved from “Precipitate the DNA“ on page 4 using either “Precipitate the proteins“ on page 5 or “Dialyse the proteins“ on page 5.

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1) Precipitate the proteins
a. Add 1.5 mL of isopropanol to the phenol-ethanol supernatant per 1 mL of YTzol Pure RNA solution used for lysis.
b. Incubate for 10 minutes.
c. Centrifuge for 10 minutes at 12,000 × *g* at 4°C to pellet the proteins.
d. Discard the supernatant with a micropipettor.

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2) Wash the proteins
a. Prepare a wash solution consisting of 0.3 M guanidine hydrochloride in 95% ethanol.

b. Resuspend the pellet in 2 mL of wash solution per 1 mL of YTzol Pure RNA solution used for lysis.
c. Incubate for 20 minutes.
Note: The proteins can be stored in wash solution for at least 1 month at 4°C or for at least 1 year at –20°C.
d. Centrifuge for 5 minutes at 7500 × *g* at 4°C.
e. Discard the supernatant with a micropipettor.
f. Repeat step 2b–step 2e twice.
g. Add 2 mL of 100% ethanol, then mix by vortexing briefly.
h. Incubate for 20 minutes.
i. Centrifuge for 5 minutes at 7500 × *g* at 4°C.
j. Discard the supernatant with a micropipettor.
k. Air dry the protein pellet for 5–10 minutes.
Note: Do not dry the pellet by vacuum centrifuge

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3) Solubilize the proteins
a. Resuspend the pellet in 200 µL of 1% SDS by pipetting up and down.
Note: To ensure complete resuspension of the pellet, we recommend that you incubate the sample at 50°C in a water bath or heat block.
b. Centrifuge for 10 minutes at 10,000 × *g* at 4°C to remove insoluble materials.
c. Transfer the supernatant to a new tube.
Proceed directly to downstream applications, or store the sample at –20°C.

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4) Determine the proteins yield
Measure protein concentration by Bradford assay.
Note: SDS concentration mush be <0.1%.

Dialyse the proteins

1. Load the phenol-ethanol supernatant into the dialysis membrane. **Note:** The phenol-ethanol solution can dissolve some types of dialysis membranes (cellulose ester, for example). Test dialysis tubing with the membrane to assess compatibility before starting.
2. Dialyze the sample against 3 changes of 0.1% SDS at 4°C. Make the first change of solution after 16 hours, the second change 4 hours later (at 20 hours), and the final change 2 hours later (at 22 hours).
Note: A SDS concentration of at least 0.1% is required to resolubilize the proteins from the pellet. If desired, the SDS can be diluted after solubilization.
3. Centrifuge the dialysate for 10 minutes at 10,000 × *g* at 4°C.
4. Transfer the supernatant containing the proteins to a new tube.
5. *(Optional)* Solubilize the pellet by adding 100 µL of 1% SDS and 100 µL of 8 M urea.
Proceed directly to downstream applications, or store the sample at – 20°C.

Troubleshooting

Observation	Possible cause	Recommended action
A lower yield than expected is observed	The samples were incompletely homogenized or lysed.	Decrease the amount of starting material.
		Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in YTzol Pure RNA solution to achieve total lysis.
	The pellet was partly solubilized	Increase the solubilization rate by pipetting the sample frequently, and heat the sample to 50–60°C.
The sample is degraded	Samples were not immediately processed or frozen after collection.	Sample must be processed or frozen immediately after collection.
	Sample preparations were stored at the incorrect temperature.	Store RNA samples at –60 to –70°C. Store DNA and protein samples at –20°C.

The RNA or DNA is contaminated	The interphase/organic phase is pipetted up with the aqueous phase.	Do not attempt to draw off the whole aqueous layer after phase separation.
	The aqueous phase is partly removed.	Remove leftovers of the aqueous phase previous to DNA precipitation.
	The DNA pellet is insufficiently washed with 0.1 M sodium citrate in 10% ethanol	Make sure pellet is washed with 0.1 M sodium citrate in 10% ethanol.
The RNA A**260/280** ratio is low	Sample was homogenized in an deficient volume of YTzol Pure RNA solution	Add the appropriate amount of YTzol Pure RNA solution for your sample type.
	The organic phase is moderately removed.	Do not attempt to draw off the entire aqueous layer after phase separation.
The DNA A**260/280** ratio is low	Phenol was not sufficiently removed from the DNA preparation.	Wash the DNA pellet one extra time in 0.1 M sodium citrate in 10% ethanol.

RNA and miRNA Isolation from Human Peripheral Blood

Reagents needed :

10x RBC Lysis Buffer
89.9 g NH4Cl , 10.0 g KHCO3 , 2.0 ml 0.5 M EDTA
Dissolve the above in approximately 800 ml ddH2O and adjust pH to 7.3. QS to 1 liter and mix thoroughly. This solution is stable for 6 months at 2 – 8° C in a tightly closed bottle.
1x RBC Lysis Buffer
Simply dilute the 10x stock solution 1:10 with ddH2O. Stable for 1 week at room temperature.
Other Reagents Needed but not supplied :
Phosphate Buffered Saline (PBS)
Isopropanol (2-propanol)
Ethanol
RNAse-free water
RNAse-Away (a cleaning solution that neutralizes RNAses on bench tops, pipettors, centrifuges, and other equipment.

Approx Yield : 30 ug RNA
Sample : Vacuette tube of human blood (equals to 8 ml)

Note: RNA is very easily degraded by ever-present RNAses. Thus, all of the tubes and solutions in this protocol must be RNase-free (autoclaving does NOT inactivate RNases).
1) Transfer contents of tube into a 50 ml polypropylene conical centrifuge tube.
2) Bring volume to 45 ml with RBC Lysis Buffer .
3) Let stand at room temperature for 10 minutes.
4) Pellet cells at 600 x g (approx 1,400 rpm) for 10 minutes in a room temp centrifuge (program #3).
5) Carefully decant supernatant.
6) Gently resuspend the pellet in 1 ml of RBC Lysis Buffer and transfer to a 1.5 ml microcentrifuge tube ; Let stand for 5 minutes.
7) Pellet cells for 2 minutes by centrifuging in a microfuge at room temperature at 3000 rpm.
8) Carefully remove the supernatant.
9) Resuspend the pellet in 1 ml of sterile DPBS.
10) Pellet cells as in step 7.

11) Carefully remove the supernatant.
12) Add 1200 µl of YTzol Pure RNA solution to each tube and resuspend the cells. Note: for a full 8 ml blood tube, the 1200 ul YTzol Pure RNA solution can be split into 2, 600 µl aliquots and frozen at -80 C until further processing.
13) Add 0.2 ml of Chloroform (CHCl3) and vortex each tube for 15 seconds, ONE AT A TIME.
14) Centrifuge the samples at 13,000 rpm for 10 minutes at 4°C.
15) Remove the upper phase and transfer to a clean microcentrifuge tube. Be careful not to remove any of the white interface when collecting the upper phase of the extraction
16) For the future collection of micro RNA (miRNA), carefully remove ~20% of the volume of the upper phase from step 16 and place into another clean, labeled, 1.5ml microfuge tube. Store this aliquot at -80 C until further processing.
17) To the remaining upper phase from step 16, add an equal volume of cold isopropanol and invert to mix.
18) The samples can be placed in a -20°C freezer to precipitate.
19) Samples are centrifuged at 13,000 rpm for 10 minutes at 4°C. Note: you may be able to see a small white pellet of RNA at the bottom of the tube after this step.
20) Carefully decant the supernatant, and rinse the pellet with 0.5 ml of ice-cold 75% ethanol. The 75% EtOH should be prepared RNase-free and stored at -20 C.
21) Centrifuge the samples at 13,000 rpm for 10 minutes at 4°C.
22) Decant the supernatant.
23) Using a pipettor, carefully remove all of the remaining liquid in the bottom of the tube.
24) Allow the pellet to dry for 5 to 10 minutes to remove any remaining ethanol.
25) Dissolve the RNA pellet by adding 20 µl of RNAse-free H2O to each sample.
26) RNA should be quantitated within 2 hours of elution. It can be kept at 4 C until that time;
it can also be held temporarily at -20 until permanent storage at -80. Repeated freeze-thaws are to be avoided, so RNA should be aliquoted for transfer as soon as possible after quantitation.