GENEzol™ TriRNA Pure Kit

For research use only

Catalogue Numbers

GZX050, GZXD050
GZX100, GZXD100
GZX200, GZXD200

Quantity

50 rxns
100 rxns
200 rxns

Introduction

The GENEzol™ TriRNA Pure Kit is a phenol and guanidine isothiocyanate plus spin column system for convenient purification of high-quality total RNA from a variety of samples. Initially, samples are homogenized in GENEzol™ Reagent without chloroform phase separation or isopropanol RNA precipitation. Following sample homogenization, simply bind, wash and elute the high-quality, total RNA in RNase-free Water and use in a variety of sensitive downstream applications.

Quality Control

The GENEzol™ TriRNA Pure Kit is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system. 10 µl from a 50 µl eluate of purified RNA is analyzed by electrophoresis on a 0.8% agarose gel.

Advantages

- Purify total RNA within 15 minutes without chloroform phase separation or isopropanol RNA precipitation
- Up to: 200 µl (blood, buffy coat, serum, plasma), 5 x 10^6 (cultured cells), 10-50 mg (tissue), 1 x 10^9 (bacteria cells), 20-50 mg (plant tissue)
- A cost effective phenol, guanidine isothiocyanate solution plus spin column system
- High quality RNA: A260/A280 >1.8, A260/A230 >1.8
- Applications: cDNA Library Construction, Cloning, RT-PCR (Endpoint), Real-Time PCR, Nuclease Protection Assays, Northern Blotting

Caution

GENEzol™ Reagent contains phenol and guanidine isothiocyanate. During operation, always work in a fume hood, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask. Disposable/non-disposable glassware, plasticware and automatic pipettes should be sterile (RNase-free) and used only for RNA procedures.

Components and Storage

Item	Volume	Product	Shipping	Storage
GENEzol™ Reagent	4 ml	GZX004/D004	room temperature	dry at 2ºC to 25ºC
	40 ml	GZX050/D050		
	80 ml	GZX100/D100		
	160 ml	GZX200/D200		
Pre-Wash Buffer[1] (Add Ethanol)	1.4 ml (0.6 ml)	GZX004/D004	room temperature	dry at room temperature (15-25ºC)
	21 ml (9 ml)	GZX050/D050		
	35 ml (15 ml)	GZX100/D100		
	70 ml (30 ml)	GZX200/D200		
DNase I[2] (2U/µl)	20 µl	GZXD004	room temperature	-20ºC
	275 µl	GZXD050		
	550 µl	GZXD100		
	550 µl x 2	GZXD200		
DNase I Reaction Buffer	200 µl	GZXD004	room temperature	dry at room temperature (15-25ºC)
	2.5 ml	GZXD050		
	5 ml	GZXD100		
	5 ml x 2	GZXD200		
Wash Buffer[3] (Add Ethanol)	2 ml (8 ml)	GZX004/D004	room temperature	dry at room temperature (15-25ºC)
	25 ml (100 ml)	GZX050/D050		
	50 ml (200 ml)	GZX100/D100		
	25 ml + 50 ml (100 ml + 200 ml)	GZX200/D200		
RNase-free Water	1 ml	GZX004/D004	room temperature	dry at room temperature (15-25ºC)
	6 ml	GZX050/D050		
	6 ml	GZX100/D100		
	15 ml	GZX200/D200		
RB Columns	4	GZX004/D004	room temperature	dry at room temperature (15-25ºC)
	50	GZX050/D050		
	100	GZX100/D100		
	200	GZX200/D200		
2 ml Collection Tubes	8	GZX004/D004	room temperature	dry at room temperature (15-25ºC)
	100	GZX050/D050		
	200	GZX100/D100		
	400	GZX200/D200		

[1, 3]Add absolute ethanol (see the bottle label for volume) to Pre-Wash Buffer and Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

[2]DNase I is shipped at room temperature and should be stored at -20ºC for extended periods after receiving the kit.

RNA Purification Protocol Procedure

Please read the entire instruction manual prior to starting the Protocol Procedure.

Additional Requirements

absolute ethanol, lysozyme and bacteria lysis buffer (bacteria only), 1.5 ml microcentrifuge tubes (RNase-free)

Optional Requirements

1 µL of 0.5M EGTA (pH=8.0) for Optional Step 2: DNA Digestion in Solution

1. Sample Homogenization and Lysis

Sample preparation should be performed at room temperature. Please follow the table below for specific sample preparation. To avoid DNA contamination of extracted RNA, be sure and use the indicated volume of GENEzol™ Reagent. Lysozyme (LY420) and Bacteria Lysis Buffer (BLB00030) can be purchased directly from Geneaid.

Sample	Procedure
Adherent Cultured Cells	1. Remove the culture medium from the culture dish. 2. **Directly add 100 µl of GENEzol™ Reagent per cm^2 of culture dish surface area**. 3. Lyse the cells directly in the culture dish by pipetting several times. 4. Incubate the sample mixture for 5 minutes at room temperature. 5. Transfer the sample to a 1.5 ml microcentrifuge tube (RNase-free).
Suspension Cultured Cells	1. **Transfer cells (up to 5 x 10^6)** to a 1.5 ml microcentrifuge tube (RNase-free). 2. Harvest by centrifugation at 300 x g for 5 minutes then remove the culture medium completely. 3. **700 µl of GENEzol™ Reagent** should be added to the cell pellet then mixed several times by pipette. 4. Incubate the sample mixture for 5 minutes at room temperature.
Tissue	1. **Excise 10-50 mg of tissue** directly from the animal or remove the tissue sample from storage. Do not use more than 50 mg of tissue per reaction. 2. **Homogenize tissue samples using one of the following methods: A.** Transfer the tissue and 700 µl of GENEzol™ Reagent to a 2 ml centrifuge tube containing ceramic beads or stainless steel beads then homogenize the sample with a TissueLyser, Disruptor Genie or similar. **B.** Transfer the tissue and 700 µl of GENEzol™ Reagent to a 1.5 ml centrifuge tube and grind the tissue with a micropestle a few times then shear the tissue by passing the lysate through a 20-G needle syringe 10 times. **C.** Transfer the tissue and 700 µl of GENEzol™ Reagent to a glass-Teflon or Polytron homogenizer. Transfer the homogenized sample to a 1.5 ml microcentrifuge tube (RNase-free). 3. Incubate the homogenized sample for 5 minutes at room temperature.
Body Fluids (blood, buffy coat, plasma, serum)	1. **Transfer up to 200 µl of liquid sample** to a 1.5 ml of microcentrifuge tube (RNase-free). 2. **Add 3 volumes of GENEzol™ Reagent per 1 volume of sample (3:1)** then mix well by vortex. 3. Incubate the sample mixture for 5 minutes at room temperature.
Bacteria	1. **Transfer bacteria cells (up to 1 x 10^9)** to a 1.5 ml microcentrifuge tube (RNase-free). 2. Centrifuge at 12-16,000 x g for 2 minutes then remove the supernatant completely. 3. **Weigh and transfer 10 mg of lysozyme powder** to a new 1.5 ml microcentrifuge tube (RNase-free). 4. **Add 1 ml of bacteria lysis buffer** to the microcentrifuge tube containing **10 mg of lysozyme**. 5. Vortex the tube until the lysozyme powder is completely dissolved. 6. **Add 100 µl of bacteria lysis buffer containing lysozyme** to the bacteria cell pellet. 7. Resuspend the cell pellet by vortex or pipetting. NOTE: Residual bacteria lysis buffer containing lysozyme should be stored at 4ºC for 2 weeks. 8. Incubate the sample for 5 minutes at room temperature. 9. Add **700 µl of GENEzol™ Reagent,** mix well by pipette then incubate at room temperature for 5 minutes.
Plant	1. **Cut off 20-50 mg of fresh or frozen plant tissue**. Do not use more than 50 mg of plant tissue per rxn. 2. **Homogenize plant tissue samples using one of the following methods: A.** Transfer the plant tissue and 700 µl GENEzol™ Reagent to a 2 ml centrifuge tube containing ceramic beads or stainless steel beads then homogenize the sample with a TissueLyser, Disruptor Genie or similar. **B.** Add liquid nitrogen to a mortar (RNase-free) and grind the plant tissue thoroughly using a pestle (RNase-free). Transfer the plant tissue powder and 700 µl of GENEzol™ Reagent to a 1.5 ml centrifuge tube then vortex briefly. 3. Incubate the homogenized sample for 5 minutes at room temperature.

2. RNA Binding

1. Centrifuge the sample at 12-16,000 x g for 1 minute to remove cell debris then transfer the clear supernatant to a new 1.5 ml microcentrifuge tube (RNase-free).

NOTE: When extracting RNA from cultured cell samples, cell debris will not commonly collect on the bottom of the microcentrifuge tube. In this case, proceed without transferring the supernatant.

2. **Add 1 volume of absolute ethanol directly to 1 volume of sample mixture (1:1) in GENEzol™ Reagent**.

3. Mix well by vortex then place a **RB Column in a 2 ml Collection Tube**.

4. **Transfer 700 µl of the sample mixture to the RB Column**. Centrifuge at 14-16,000 x g for 1 minute then discard the flow-through.

5. Repeat the RNA Binding Step by transferring the remaining sample mixture to the **RB Column**.

6. Centrifuge at 14-16,000 x g for 1 minute then discard the flow-through. Place the **RB Column** in a new **2 ml Collection Tube**.

Optional Step 1: In Column DNase I Digestion

IMPORTANT

DNA contamination is significantly reduced following In Column DNase I Digestion. However, traces of residual DNA may be detected in very sensitive applications. In this situation, please perform Optional Step 2: DNA Digestion In Solution instead to efficiently remove trace amounts of DNA. Standard DNase buffers are incompatible with In Column DNase I Digestion and may affect RNA integrity and reduce yield.

1. Add **400 µl of Wash Buffer (make sure ethanol was added) to the RB Column** then centrifuge at 14-16,000 x g for 30 seconds.
2. Discard the flow-through and place the **RB Column** back in the **2 ml Collection Tube**.
3. Prepare DNase I solution in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

DNase I	5 µl (2 U/µl)
DNase I Reaction Buffer	45 µl
Total volume	50 µl

4. Gently pipette the DNase I solution to mix (DO NOT vortex) then add **DNase I solution (50 µl)** into the **CENTER** of the **RB column** matrix.
5. Incubate the column for 15 minutes at room temperature (20-30ºC) then proceed with RNA Wash.

3. RNA Wash

1. **Add 400 µl of Pre-Wash Buffer (make sure ethanol was added)** to the **RB Column** then centrifuge at 14-16,000 x g for 30 seconds.
2. Discard the flow-through then place the **RB Column** back in the **2 ml Collection Tube**.
3. **Add 600 µl of Wash Buffer (make sure ethanol was added)** to the **RB Column**.
4. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the **RB Column** back in the **2 ml Collection Tube**.
5. **Add 600 µl of Wash Buffer (make sure ethanol was added)** to the **RB Column**.
6. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through.
7. Place the **RB Column** back in the **2 ml Collection Tube**.

NOTE: For blood samples only, wash the RB Column again with 600 µl of Wash Buffer.

8. Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.

4. RNA Elution

1. Place the dry **RB Column** in a clean 1.5 ml microcentrifuge tube (RNase-free).
2. **Add 25-50 µl of RNase-free Water** into the **CENTER** of the column matrix.
3. Let stand for at least 3 minutes to ensure the **RNase-free Water** is completely absorbed by the matrix.
4. Centrifuge at 14-16,000 x g for 1 minute to elute the purified RNA.

Optional Step 2: DNA Digestion In Solution

1. Prepare DNase I reaction in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

RNA in RNase-free water	1-40µl
DNase I	0.5 µl/ µg RNA
DNase I Reaction Buffer	5 µl
RNase-free water	add to final volume = 50 µl
Total volume	50 µl

2. Gently pipette the DNase I reaction solution to mix (DO NOT vortex) then incubate the microcentrifuge tube at 37ºC for 15-30 minutes.
3. Stop the reaction by adding 1 µl of 0.5M EGTA (pH=8.0) then incubate the microcentrifuge tube at 75°C for 10 minutes.

NOTE: DNase I Reaction Buffer may cause aberrant migration or smearing of RNA on gels. If analyzing RNA by gel electrophoresis, repurify the RNA sample by using the Geneaid™ RNA Cleanup Kit instead of stopping the reaction with EGTA and heat inactivation.

GENEzol™ TriRNA Pure Kit Functional Test Data (HeLa Cells)

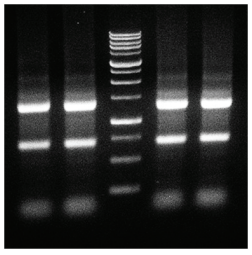

Figure 1. RNA was purified using the GENEzol™ TriRNA Pure Kit in parallel to the similar product from competitor Z. 5 x 10^5 HeLa cells were homogenized using GENEzol™ Reagent and competitor Z tri reagent. RNA was then purified using the corresponding kits spin column procedure. 10 µl from a 50 µl eluate of purified RNA was analyzed by electrophoresis on a 0.8% agarose gel.

Product Test	ng/µl	260/280	260/230	Yield
1. Competitor Z	162.5	2.00	2.07	8.1 µg
2. Competitor Z	160.7	2.03	2.07	8.0 µg
3. Geneaid	164.0	2.00	2.07	8.2 µg
4. Geneaid	161.6	2.03	2.06	8.0 µg

GENEzol™ TriRNA Pure Kit Real-Time PCR Data

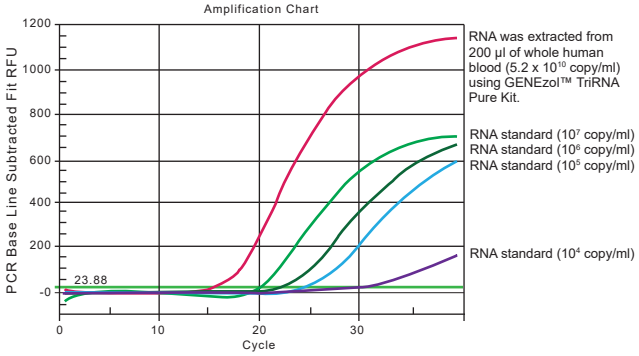

Figure 2. Quantitative analysis of human beta globin mRNA extracted by GENEzol™ TriRNA Pure Kit using a Taqman probe 1-step qRT-PCR assay. The assay was run on a BioRad IQ5 thermal cycler. The high yield, high quality extracted RNA was amplified quickly following a very short C_T (threshold cycle) compared to the RNA standards.

Troubleshooting

Problem	Cause	Solution
Low Yield	A. Sample lysis or homogenization was incomplete B. Incorrect RNA elution C. Precipitates may form during the RNA binding step after adding 1 volume of absolute ethanol to the sample mixture in GENEzol™ Reagent if too much sample material is used	A. Starting material should be reduced and completely dissolved in GENEzol™ Reagent. B. Make sure RNase-free Water is added to the center of the RB Column and is absorbed completely. C. Reduce the sample amount to half of the original.
Degraded RNA	A. Incorrect sample preparation and/or storage B. Incorrect storage temperature	A. Process or freeze samples immediately after collection. B. Extracted RNA should be stored at -70ºC.
Low RNA A260/A280	A. Volume of GENEzol™ Reagent was insufficient for proper sample homogenization B. Incomplete wash step	A. Volume of GENEzol™ Reagent is sample dependent and should be added according to the sample homogenization specifications. B. Wash the RB Column with ethanol added Wash Buffer 3 times.
Eluted RNA does not perform well in downstream applications	A. Residual ethanol contamination	A. Following the wash step, dry the RB Column with additional centrifugation at 14-16,000 x g for 5 minutes or incubate at 60°C for 5 minutes.
Samples were stored in 1 ml of tri-reagent in a 1.5 ml microcentrifuge tube	A. 1 ml (1 volume) of absolute ethanol cannot be added to the same 1.5 ml microcentrifuge tube	A. Following centrifugation to remove insoluble cell debris, transfer the supernatant to a 2 ml or 15 ml centrifuge tube (RNase-free) and add 1 volume of absolute ethanol then mix well by vortex. Transfer 700 µl of the sample mixture to the RB Column then centrifuge and discard the flow-through. Repeat the RNA Binding step until all of the sample mixture has been passed through the RB Column.

Related RNA/DNA Purification and Extraction Products

Total RNA Purification		
Product	Package Size	Catalogue Number
Total RNA Mini Kit (Blood/Cultured Cell)	50/100/300 preps	RB050/100/300
Total RNA Mini Kit (Tissue)	50/100/300 preps	RT050/100/300
Total RNA Mini Kit (Plant)	50/100/300 preps	RP050/100/300
Presto™ Mini RNA Bacteria Kit	50/100/300 preps	RBB050/100/300
Presto™ Mini RNA Yeast Kit	50/100/300 preps	RBY050/100/300
miRNA Isolation Kit	50/100 preps	RMI050/100
GENEzol™ Reagent	100/200 rxns	GZR050/100/200
GENEzol™ TriRNA Bacteria Kit	50/100/200 rxns	GZB050/100/200
GENEzol™ TriRNA Pure Kit	50/100/200preps	GZX050/100/200, GZXD050/100/200
TriRNA Pure Kit	50/100/200 preps	TRP050/100/200, TRPD050/100/200
RNA Cleanup Kit	50/100 preps	PR050/100
GENEzol™96 Well TriRNA Pure Kit	4/10 x 96 preps	96GZX04/10
Genomic DNA Purification		
Product	Package Size	Catalogue Number
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/300
Genomic DNA Midi Kit (Blood/Cultured Cell)	25 preps	GDI25
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	GDM10/25
Genomic DNA Mini Kit (Tissue)	50/100/300 preps	GT050/100/300
gSYNC™ DNA Extraction Kit	100/300 preps	GS100/300
Genomic DNA Mini Kit (Plant)	100 preps	GP100
Genomic DNA Maxi Kit (Plant)	10/25 preps	GPM10/25
Geneaid™ DNA Isolation Kit (Blood)	100/1,000 rxns	GEB100/01K(+)
Geneaid™ DNA Isolation Kit (Bacteria)	150/1,500rxns	GEE150/1.5K(+)
Geneaid™ DNA Isolation Kit (Tissue)	150/1,500 rxns	GET150/1.5K(+)
Geneaid™ DNA Isolation Kit (Cultured Cell)	150/1,500 rxns	GEC150/1.5K(+)
GENEzol™ DNA Reagent Plant	100/200 rxns	GR100/200
Presto™ Mini gDNA Yeast Kit	100/300 preps	GBY100/300
Presto™ Mini gDNA Bacteria Kit	100/300 preps	GBB100/101/300/301
Geneius™ Micro DNA Extraction Kit	100/300 preps	GMB100/300
Presto™ Buccal Swab gDNA Extraction Kit	100/300 preps	GSK100/300
Presto™ 96 Well Blood Genomic DNA Extraction Kit	4/10 x 96 preps	96GBP04/10