GENEzol™ TriRNA Bacteria Kit

For research use only

Catalogue Numbers

GZB050, GZBD050
GZB100, GZBD100
GZB200, GZBD200

Quantity

50 rxns
100 rxns
200 rxns

Geneaid

CERTIFICATE NO. QAIC/TW/50077
ISO 9001:2008 QMS

Introduction

The GENEzol™ TriRNA Bacteria Kit is a phenol and guanidine isothiocyanate plus spin column system for convenient purification of high-quality total RNA from bacteria samples. Bacterial cell walls are initially lysed using Lysozyme. The sample is then 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 Bacteria Kit is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system. 5 µl from a 50 µl eluate of purified RNA is analyzed by electrophoresis on a 1% agarose gel.

Advantages

- Purify total RNA within 20 minutes without chloroform phase separation or isopropanol RNA precipitation
- Up to: 1 x 10^9 bacteria cells
- 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

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	GZB004/D004	room temperature	dry at 2ºC to 25ºC
	40 ml	GZB050/D050		
	80 ml	GZB100/D100		
	160 ml	GZB200/D200		
Pre-Wash Buffer[1] (Add Ethanol)	1.4 ml (0.6 ml)	GZB004/D004	room temperature	dry at room temperature (15-25ºC)
	21 ml (9 ml)	GZB050/D050		
	35 ml (15 ml)	GZB100/D100		
	70 ml (30 ml)	GZB200/D200		
Lysozyme[2]	8 mg	GZB004/D004	room temperature	-20ºC
	55 mg	GZB050/D050		
	110 mg	GZB100/D100		
	250 mg	GZB200/D200		
Bacteria Lysis Buffer	1.5 ml	GZB004/D004	room temperature	dry at room temperature (15-25ºC)
	15 ml	GZB050/D050		
	15 ml	GZB100/D100		
	30 ml	GZB200/D200		
DNase I[3] (2U/µl)	20 µl	GZBD004	room temperature	-20ºC
	275 µl	GZBD050		
	550 µl	GZBD100		
	550 µl x 2	GZBD200		
DNase I Reaction Buffer	200 µl	GZBD004	room temperature	dry at room temperature (15-25ºC)
	2.5 ml	GZBD050		
	5 ml	GZBD100		
	5 ml x 2	GZBD200		
Wash Buffer[4] (Add Ethanol)	2 ml (8 ml)	GZB004/D004	room temperature	dry at room temperature (15-25ºC)
	25 ml (100 ml)	GZB050/D050		
	50 ml (200 ml)	GZB100/D100		
	25 ml + 50 ml (100 ml + 200 ml)	GZB200/D200		
RNase-free Water	1 ml	GZB004/D004	room temperature	dry at room temperature (15-25ºC)
	6 ml	GZB050/D050		
	6 ml	GZB100/D100		
	15 ml	GZB200/D200		
RB Columns	4	GZB004/D004	room temperature	dry at room temperature (15-25ºC)
	50	GZB050/D050		
	100	GZB100/D100		
	200	GZB200/D200		
2 ml Collection Tubes	8	GZB004/D004	room temperature	dry at room temperature (15-25ºC)
	100	GZB050/D050		
	200	GZB100/D100		
	400	GZB200/D200		

[1, 4] 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, 3] DNase I and Lysozyme are 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, 1.5 ml microcentrifuge tubes (RNase-free)

Optional Requirements

1 µL of 20 mM 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. To avoid DNA contamination of extracted RNA, be sure and use the indicated volume of GENEzol™ Reagent.

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 **Lysozyme powder (1 mg/sample)** to a new 1.5 ml microcentrifuge tube (RNase-free).
4. Add **Bacteria Lysis Buffer (100 µl/sample)** to the microcentrifuge tube containing **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.
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.

2. RNA Binding

1. Add **700 µl of absolute ethanol** directly to the sample mixture.
2. Mix well by vortex then place a **RB Column in a 2 ml Collection Tube**.
3. Transfer **700 µl of the sample mixture to the RB Column**.
4. 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.
7. 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. 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** then 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	1-40 µl
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 20 mM EGTA (pH=8.0) then incubate the microcentrifuge tube at 65°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.

GENEzol™ TriRNA Bacteria Kit Functional Test Data

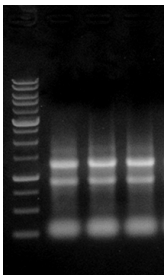

Figure 1. RNA was extracted using the GENEzol™ TriRNA Bacteria Kit. An *Escherichia coli* (1×10^9) culture (OD600=2, 1 ml) was harvested by centrifugation at 16,000 x g for 2 minutes, followed by RNA extraction. 5 µl from a 50 µl eluate of RNA was analyzed by electrophoresis on a 1% agarose gel. M = Geneaid 1 Kb DNA Ladder

Test	RNA Concentration	260/280	260/230	Yield
1	502.6 µg/ml	2.06	2.18	25.13 µg
2	518.3 µg/ml	2.07	2.21	25.92 µg
3	506.0 µg/ml	2.08	2.24	25.30 µg

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.

Related DNA/RNA Purification and Extraction Products

Plasmid DNA Purification		
Product	Package Size	Catalogue Number
Presto™ Mini Plasmid Kit	100/300 preps	PDH100/300
Presto™ Midi Plasmid Kit	25 preps	PIF025
Presto™ Midi Plasmid Kit (Endotoxin Free)	25 preps	PIFE25
Large Plasmid DNA Extraction Kit	100/300 preps	PDL100/300
Midiprep Spin Column Plasmid Kit	25 preps	PA025
Geneaid™ Plasmid Midi Kit	25 preps	PI025
Geneaid™ Plasmid Midi Kit (Endotoxin Free)	25 preps	PIE25
Presto™ Plasmid DNA Concentration Kit	250/500/1000 preps	PC0250/500/1000
Geneaid™ Plasmid Maxi Kit	10/25 preps	PM010/25
Geneaid™ Plasmid Maxi Kit (Endotoxin Free)	10/25 preps	PME10/25
Presto™ 96 Well Plasmid Kit	4/10 x 96 preps	96PDV04/10, 96PDC04/10
Presto™ Plasmid 96 Well Binding Plate	10 plates	96PBP01
Presto™ Plasmid 96 Well FIlter Plate	10 plates	96PFP01
Post Reaction DNA Purification		
Product	Package Size	Catalogue Number
GenepHlow™ Gel Extraction Kit	100/300 preps	DFG100/300
GenepHlow ™ PCR Cleanup Kit	100/300 preps	DFC100/300
GenepHlow ™ Gel/PCR Kit	100/300 preps	DFH100/300
GenepHlow ™ DNA Cleanup Maxi Kit	10/25 preps	DFM010/025
Small DNA Fragments Extraction Kit	100/300 preps	DF101/301
Presto™ Max Gel/PCR Kit (Large DNA Fragments)	100/300 preps	DFL100/300
Presto™ 96 Well PCR Cleanup Kit	4/10 x 96 preps	96DFH04/10
Presto™ 96 Well Gel Extraction Kit	4/10 x 96 preps	96DFG04/10
Presto™ PCR Cleanup Kit 96 Well Binding Plate	10 plates	96DBP01
DNA Cleanup Kit	100/300 preps	DP100/300
G-25 Gel Filtration Desalting Column	50 rxns	CG025
G-50 Gel Filtration Dye Terminator Removal Column	50 rxns	CG050
96-Well G-50 Gel Filtration Plate	4/10 x 96 rxns	CGP04/10
Gel Extraction Tool	25 pcs	GXT025
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)	150/1,500 rxns	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
DNA RNA Purification		
Product	Package Size	Catalogue Number
Presto™ DNA/RNA/Protein Extraction Kit	50/100 preps	DRP050/100
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 rxns	GZB050/100
GENEzol™ TriRNA Pure Kit	50/100/200 preps	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