IBI SCIENTIFIC

Instruction Manual
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For Research Use Only

rBAC Mini RNA Bacteria Kit

IB47420 (4 Preparation Sample Kit)
IB47421 (100 Preparation Kit)
IB47422 (300 Preparation Kit)

Advantages

Sample: Gram (+) positive and Gram (-) negative bacterial cells
Yield: up to 60 µg of RNA (1 x 10^9 *Escherichia coli*: 40-45 µg, 1 x 10^9 *Bacillus subtilis*: 50-55 µg)
Convenient: includes Lysozyme and Bacteria Lysis Buffer
Format: certified DNase and RNase-free spin columns
Time: within 20 minutes
Elution Volume: 50-100 µl
Kit Storage: dry at room temperature (15-25°C), DNase I and Lysozyme are shipped at room temperature and should be stored at -20°C for extended periods

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Introduction

The rBAC Mini RNA Bacteria Kit was designed for total RNA purification from Gram (-) negative and Gram (+) positive bacteria. The provided Lysozyme and Bacteria Lysis Buffer will efficiently lyse bacterial cell walls consisting of the peptidoglycan layer. Detergents and chaotropic salt are used to further lyse cells and inactivate RNase while RNA is bound by the glass fiber matrix of the RNA spin column. Once any contaminants have been removed, using the Wash Buffer (containing ethanol), the purified total RNA is eluted by RNase-free Water and is ready for use in a variety of subsequent reactions.

Quality Control

The quality of the rBAC Mini RNA Bacteria Kit is tested on a lot-to-lot basis by isolating RNA from *Escherichia coli* (1×10^9) culture (OD600=1.3, 1 ml) harvested by centrifugation at 16,000 x g for 1 minute. 10 µl from a 50 µl eluate of purified RNA is analyzed by electrophoresis on a 0.8% agarose gel.

Kit Components

Component	IB47420	IB47421	IB47422
Bacteria Lysis Buffer	1.5 ml	30 ml	75 ml
Lysozyme[1]	8 mg	250 mg	610 mg
RB Buffer	2 ml	60 ml	130 ml
DNase I[2] (2U/µl)	20 µl	550 µl	550 µl x 3
DNase I Reaction Buffer	200 µl	5 ml	15 ml
W1 Buffer	2 ml	50 ml	130 ml
Wash Buffer[3] (Add Ethanol)	1.5 ml (6 ml)	25 ml + 12.5 ml (100 ml) (50 ml)	50 ml x 2 (200 ml x 2)
RNase-free Water	1 ml	15 ml	30 ml
RB Columns	4	100	300
2 ml Collection Tubes	8	200	600

[1,2]Lysozyme and DNase I are shipped at room temperature and should be stored at -20ºC for extended periods after receiving the kit.

[3]Add absolute ethanol (see the bottle label for volume) to 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.

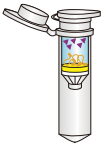
Steps to prevent RNase contamination

1. During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.
2. Disposable plasticware and automatic pipettes should be sterile (RNase-free) and used only for RNA procedures.
3. Non-disposable glassware or plasticware should also be sterile (RNase-free).

During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

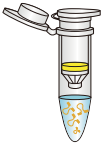
Quick Protocol Diagram

Cell lysis of bacteria samples



RNA binding to membrane while contaminants remain suspended

Wash (removal of contaminants while RNA remains bound to membrane)



Elution of pure total RNA which is ready for subsequent reactions

rBAC Mini RNA Bacteria Kit Protocol

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

IMPORTANT BEFORE USE!

Add absolute ethanol (see the bottle label for volume) to 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.

DNA Removal Options: For DNA-free RNA perform either option 1 (following RNA Binding) or option 2 (following RNA Elution)·

Additional Requirements

absolute ethanol, ddH_2O (RNase-free and DNase-free) to prepare 70% ethanol, microcentrifuge tubes (RNase-free), pipette tips (RNase-free), ß-mercaptoethanol, 15 ml centrifuge tube (RNase-free)

Bacteria Protocol Procedure

1. Sample Preparation

Transfer **bacterial cells (up to 1 x 10^9) to a 1.5 ml microcentrifuge tube (RNase-free)**. Centrifuge for 1 minute at 14-16,000 x g then remove the supernatant completely. Transfer required volume of **Bacteria Lysis Buffer (200 µl/sample) to a 15 ml centrifuge tube (RNase-free)**. Add **Lysozyme (2 mg/200 µl) to Bacteria Lysis Buffer (in the 15 ml centrifuge tube)** and vortex to completely dissolve the Lysozyme. Transfer **200 µl of Bacteria Lysis Buffer (make sure Lysozyme was added)** to the sample in the 1.5 ml microcentrifuge tube then re-suspend the pellet by pipetting. Incubate at room temperature for 10 minutes. During incubation, invert the tube every 2-3 minutes.

2. Cell Lysis

Add **300 µl of RB Buffer and 3 µl ß-mercaptoethanol (or 6 µl of freshly prepared 2M Dithiothreitol in RNase Free Water)** and vortex. Incubate at room temperature for 5 minutes then centrifuge at 14-16,000 x g for 2 minutes. Transfer the supernatant to a new 1.5 ml microcentrifuge tube (RNase-free).

3. RNA Binding

Add **500 µl of 70% ethanol to the lysate** and pipette immediately. Place a **RB Column** in a 2 ml Collection Tube. **Transfer 500 µl of the mixture to the RB Column**. Centrifuge at 14-16,000 x g for 1 minute then discard the flow-through. Transfer the remaining mixture to the same **RB Column** and centrifuge at 14-16,000 x g for 1 minute. Discard the flow-through and place the RB Column in a new 2 ml Collection Tube.

Optional Step 1: In Column DNase I Digestion

The amount of 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 effect 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 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 Step 4 RNA Wash.

4. RNA Wash

Add **400 µl of W1 Buffer to the RB Column** then centrifuge at 14-16,000 x g for 1 minute. Discard the flow-through and place the **RB Column** back in the 2 ml Collection Tube. **Add 600 µl of Wash Buffer (make sure ethanol was added)** into the **RB Column**. Centrifuge at 14-16,000 x g for 1 minute. Discard the flow-through then place the **RB Column** back in the 2 ml Collection Tube. Add **600 µl of Wash Buffer (make sure ethanol was added)** into the **RB Column**. Centrifuge at 14-16,000 x g for 1 minute. Discard the flow-through then place the **RB Column** back in the 2 ml Collection Tube. Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.

5. RNA Elution

Place the **dried RB Column** in a clean 1.5 ml microcentrifuge tube (RNase-free). **Add 50 µl of RNase-free Water** into the **CENTER** of the column matrix. Let stand for at least 3 minutes to ensure the **RNase-free Water** is absorbed by the matrix. 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 RNA Pure Kit instead of stopping the reaction with EGTA.

Troubleshooting

Low Yield

Clogged Column.

Reduce the amount of starting material or separate it into multiple tubes. Centrifugation temperature must be between 20ºC to 25ºC. Bacteria cells were not completely homogenized. Make sure Lysozyme was added to Bacteria Lysis Buffer immediately prior to use.

Residual Ethanol Contamination.

Following the wash step, dry the RB Column with additional centrifugation at 14-16,000 x g for 5 minutes.

RNA Degradation.

The harvested sample should be stabilized immediately prior to use. Disposable plasticware and automatic pipettes should be sterile (RNase-free) and used only for RNA procedures. Non-disposable glassware or plasticware should also be sterile (RNase-free).

rBAC Mini RNA Bacteria Kit Functional Test Data

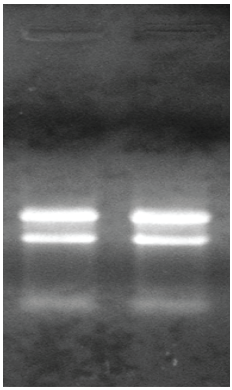


Figure 1. RNA was extracted using the rBAC Mini RNA Bacteria Kit. An *Escherichia coli* (1×10^9) culture (OD600=1.3, 1 ml) was harvested by centrifugation at 16,000 x g for 1 minute. 10 µl from a 50 µl eluate of purified RNA was analyzed by electrophoresis on a 0.8% agarose gel.

Test	RNA Yield	260/280	260/230
1	41.56 µg	2.14	2.35
2	40.87 µg	2.15	2.32

Related RNA/DNA Extraction Products

Total RNA Extraction		
Product	Package Size	Catalogue Number
Total RNA Mini Kit (Blood/Cell)	50/100/300 preps	IB47321/322/323
Total RNA Mini Kit (Tissue)	50/100/300 preps	IB47301/302/303
Total RNA Mini Kit (Plant)	50/100/300 preps	IB47341/342/343
rBAC Mini RNA Bacteria Kit	100/300 preps	IB47421/422
rYeast Total RNA Mini Kit	100/300 preps	IB47411/412
miRNA Isolation Kit	100 preps	IB47371
IBI Isolate	100/200 rxns	IB47601/302
Plasmid DNA Extraction		
Product	Package Size	Catalogue Number
I-Blue Mini Plasmid Kit	100/300 preps	IB47171/172
I-Blue Mini Plasmid Endo Free Kit	100 preps	IB47176
I-Blue Midi Plasmid Kit	25 preps	IB47181
I-Blue Midi Plasmid Kit (Endotoxin Free)	25 preps	IB47191
Midi Fast Ion Plasmid Kit	25 preps	IB47111
Midi Fast Ion Plasmid Kit (Endotoxin Free)	25 preps	IB47113
Maxi Fast Ion Plasmid Kit	10/25 preps	IB47121/122
Maxi Fast Ion Plasmid Kit (Endotoxin Free)	10/25 preps	IB47124/125
96 Well Plasmid Kit	4/10 x 96 preps	IB47151/152
Post Reaction DNA Extraction		
Product	Package Size	Catalogue Number
Gel/PCR DNA Fragments Extraction Kit	100/300 preps	IB47020/030
Small DNA Fragments Extraction Kit	100/300 preps	IB47061/062
96 Well PCR Cleanup Kit	4/10 x 96 preps	IB47040/050
Genomic DNA Extraction		
Product	Package Size	Catalogue Number
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	IB47201/202
Genomic DNA Mini Kit (Tissue)	50/300 preps	IB47221/222
gMAX Mini Kit (Blood/Tissue)	100/300 preps	IB47281/282
Genomic DNA Mini Kit (Plant)	100 preps	IB47230
gSWAB Mini Genomic DNA Kit	100/300 preps	IB47276/277
gBAC Mini DNA Bacteria Kit	100/300 preps	IB47291/292
gYEAST Genomic DNA Kit	100/300 preps	IB47266/267
96 Well Blood Genomic DNA Extraction Kit	4/10 x 96 preps	IB47251/252
gPURE Cell DNA Isolation Kit	100/1000 rxns	IB47431/432
DNA/RNA/Protein Extraction		
Product	Package Size	Catalogue Number
DNA/RNA/Protein Extraction Kit	50/100 preps	IB47701/702

For additional product information please visit www.ibisci.com. Thank you!

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