For Research Use Only

IBI SCIENTIFIC

INSTRUCTION MANUAL

IBI Tri-Isolate

IB47630 (4 rxns)
IB47631 (100 rxns)
IB47632 (200 rxns)

Catalogue Numbers	Quantity
IB47630	4 rxns
IB47631	100 rxns
IB47632	200 rxns

Additional Requirements for Bacteria RNA Extraction

IB47633 Bacteria Lysis Kit - 100 rxns, IB47634 Bacteria Lysis Kit - 200 rxns

Introduction

IBI Tri-Isolate is a phenol and guanidine isothiocyanate plus spin column system for convenient purification of high-quality total RNA froma variety of samples. Initially, samples are homogenized in IBI Isolate, treated with chlorform, then the aqueous phase is transfered to a binding column. Following phase separation, simply bind, wash and elute the high-quality, total RNA in RNase-free Water and use in a variety of sensitive down-stream applications.

Quality Control

IBI Tri-Isolate is tested on a lot-to-lot basis according to IBI'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

- 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

IBI Isolate 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
IBI Isolate	4 ml	IB47630	room temperature	dry at 2°C to 25°C
	80 ml	IB47631		
	160 ml	IB47632		
Pre-Wash Buffer[1] (Add Ethanol)	1.4 ml (0.6 ml)	IB47630	room temperature	dry at room temperature (15-25°C)
	35 ml (15 ml)	IB47631		
	70 ml (30 ml)	IB47632		
DNase I[2] (2U/µl)	20 µl	IB47630	room temperature	-20°C
	550 µl	IB47631		
	550 µl x 2	IB47632		
DNase I Reaction Buffer	220 µl	IB47630	room temperature	dry at room temperature (15-25°C)
	5 ml	IB47631		
	5ml x 2	IB47632		
Wash Buffer[3] (Add Ethanol)	2 ml (8 ml)	IB47630	room temperature	dry at room temperature (15-25°C)
	50 ml (200 ml)	IB47631		
	25 ml + 50 ml (100 ml + 200 ml)	IB47632		
RNase-free Water	1 ml	IB47630	room temperature	dry at room temperature (15-25°C)
	6 ml	IB47631		
	15 ml	IB47632		
RB Columns	4	IB47630	room temperature	dry at room temperature (15-25°C)
	100	IB47631		
	200	IB47632		
2 ml Collection Tubes	8	IB47630	room temperature	dry at room temperature (15-25°C)
	200	IB47631		
	400	IB47632		

[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 -20°C for extended periods after receiving the kit.

Bacteria Lysis Kit	Volume	Product	Shipping	Storage
Lysozyme[1]	110 mg	IB47633	room temperature	-20°C
	250 mg	IB47634		
Bacteria Lysis Buffer	15 ml	IB47633	room temperature	dry at room temperature (15-25°C)
	30 ml	IB47634		

[1]Lysozyme is shipped at room temperature and should be stored at -20°C for extended periods after receiving the kit.

RNA Purification Protocol Procedur

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), chloroform.

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. Please follow the table below for specific sample preparation. To avoid DNA contamination of extracted RNA, be sure and use the indicated volume of IBI Isolate.

Sample	Procedure
Adherent Cultured Cells	1. Remove the culture medium from the culture dish. 2. **Directly add 100 µl of IBI Isolate 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 IBI Isolate** 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 IBI Isolate 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 IBI Isolate 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 IBI Isolate 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 IBI Isolate per 1 volume of sample (3:1)** then mix well by vortex. 3. Incubate the sample mixture for 5 minutes at room temperature.
Bacteria (The Bacteria Lysis Kit is an additional requirement for this step)	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 4°C for 2 weeks. 8. Incubate the sample for 5 minutes at room temperature. 9. Add **700 µl of IBI Isolate**, 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 tissu and 700 µl IBI Isolate 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 IBI Isolate 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 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 **140 µl of chloroform and shake the tube vigorously for 10 seconds then let stand for 2 minutes at room temperature.**
3. Centrifuge at 12-16,000 x g for 5 minutes at 4ºC (then heat the centrifuge to room temperature if used for the following steps).
4. Transfer **350 µl of the upper aqueous phase** to a new 1.5 ml microcentrifuge tube (RNase-free).
5. Add **350 µl of absolute ethanol** and mix well by vortex.
6. Place a **RB Column in a 2 ml Collection Tube** and transfer **all of the sample mixture to the RB Column**.
7. Centrifuge at 14-16,000 x g for 1 minute then discard the flow-through.
8. 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.

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**.

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	1-40 µl
DNase I	0.5 µl/µg RNA
DNase I Reaction Buffer	5µl
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 65ºC 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.

IBI Plant Isolate Functional Test Data

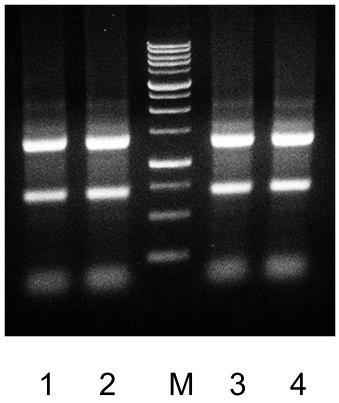

Figure 1. RNA was purified using IBI Tri-Isolate in parallel to the similar product from competitor Z. 5 x 10^5 HeLa cells were homogenized using IBI Isolate and competitor Z tri reagent. RNA was then purified using the corresponding kits spin column procedure. 10 µl from a 50 µl eluate of purified RNA 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. IBI	164.0	2.00	2.07	8.2 µg
4. IBI	161.6	2.03	2.06	8.0 µg

IBI Tri-Isolate Real-Time PCR Data

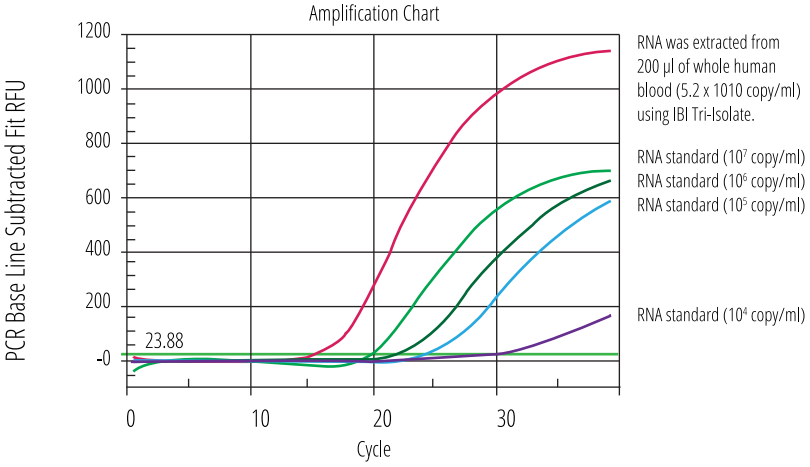

Figure 2. Quantitative analysis of human beta globin mRNA extracted by IBI Tri-Isolate using a Taqman probe 1-step qRT-PCR assay. The assay was run on a BioRad IQ5 thermal cycler. The high yield, high quality extracted RNA was amplified quickly following a very short C_T (threshold cycle) compared to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

Troubleshooting

Problem	Volume	Shipping
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 sample in IBI Isolate if too much sample material is used.	**A**. Starting material should be reduced and completely dissolved in IBI Isolate. **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 -70°C.
Low RNA A260/A280	**A**. Volume of IBI Isolate was insufficient for proper sample homogenization. **B**. Incomplete wash step.	**A**. Volume of IBI Isolate is sample dependent and should be added according to the sample homogenization specifications. **B**. Wash 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 thesame 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 flowthrough. 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

RNA Extraction and Purification		
Product	Package size	Catalogue number
Total RNA Mini Kit (Blood/Cultured 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	IB47601/602
IBI Tri-Isolate	100/200 rxns	IB47631/632
RNA Pure Kit	50/100 rxns	IB47641/642

Virus DNA/RNA Purification		
Product	Package size	Catalogue number
Viral Nucleic Acid Extraction Kit II	50/100/300 preps	IB47401/402/403

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

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