IBI SCIENTIFIC

INSTRUCTION MANUAL

Isolate 96 Well RNA Pure Kit

For Research Use Only

IB47635 (2 x 96 well plates/kit)
IB47636 (4 x 96 well plates/kit)
IB47637 (10 x 96 well plates/kit)

Advantages

Sample:	up to 2 x 10^6 cultured cells, 10-20 mg of animal tissue, up to 150 µl of body fluids, up to 1 x 10^9 bacteria cells, 10-20 mg of plant tissue per well
Binding Capacity:	50 µg of RNA per well
Format:	Isolate Reagent combined with RNA 96 Well Binding Plate
Operation Time:	60 minutes
Elution Volume:	60-80 µl (dead volume: 20-25 µl)
Kit Storage:	dry at room temperature (15-25°C)

Introduction

The Isolate 96 Well RNA Pure Kit is a phenol and guanidine isothiocyanate plus 96 well RNA binding plate system for high-throughput purification of high-quality total RNA from a variety of samples. Initially, samples are homogenized in Isolate 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 quality of the Isolate 96 Well RNA Pure Kit 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.

Kit Contents

Component	IB47635	IB47636	IB47637
Isolate Reagent	100 ml	200 ml	250 ml x 2
Pre-Wash Buffer [1] (Add Ethanol)	70 ml (30 ml)	70 ml x 2 (30 ml x 2)	175 ml x 2 (75 ml x 2)
Wash Buffer [2] (Add Ethanol)	50 ml x 2 (200 ml x 2)	50 ml x 4 (200 ml x 4)	50 ml x 10 (200 ml x 10)
RNase-Free Water	30 ml	15 ml x 1 30 ml x 1	30 ml x 3
DNase I (2U/µl)[3]	1 ml	1 ml x 2	1 ml x 5
DNase I Reaction buffer	5 ml x 2	15 ml x 2	15 ml x 4
RNA 96 Well Binding Plates	2	4	10
Microtubes (Racked)	2	2	2
Microtubes (8-strip)	12 x 2	12 x 6	12 x 18
Caps for Microtubes (8-stip)	48	48 x 2	48 x 5
96 Deep Well Plates[4]	2	2	2
0.35 ml Collection Plates	2	4	10
Adhesive Film	2	4	10

[1] [2] 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.

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

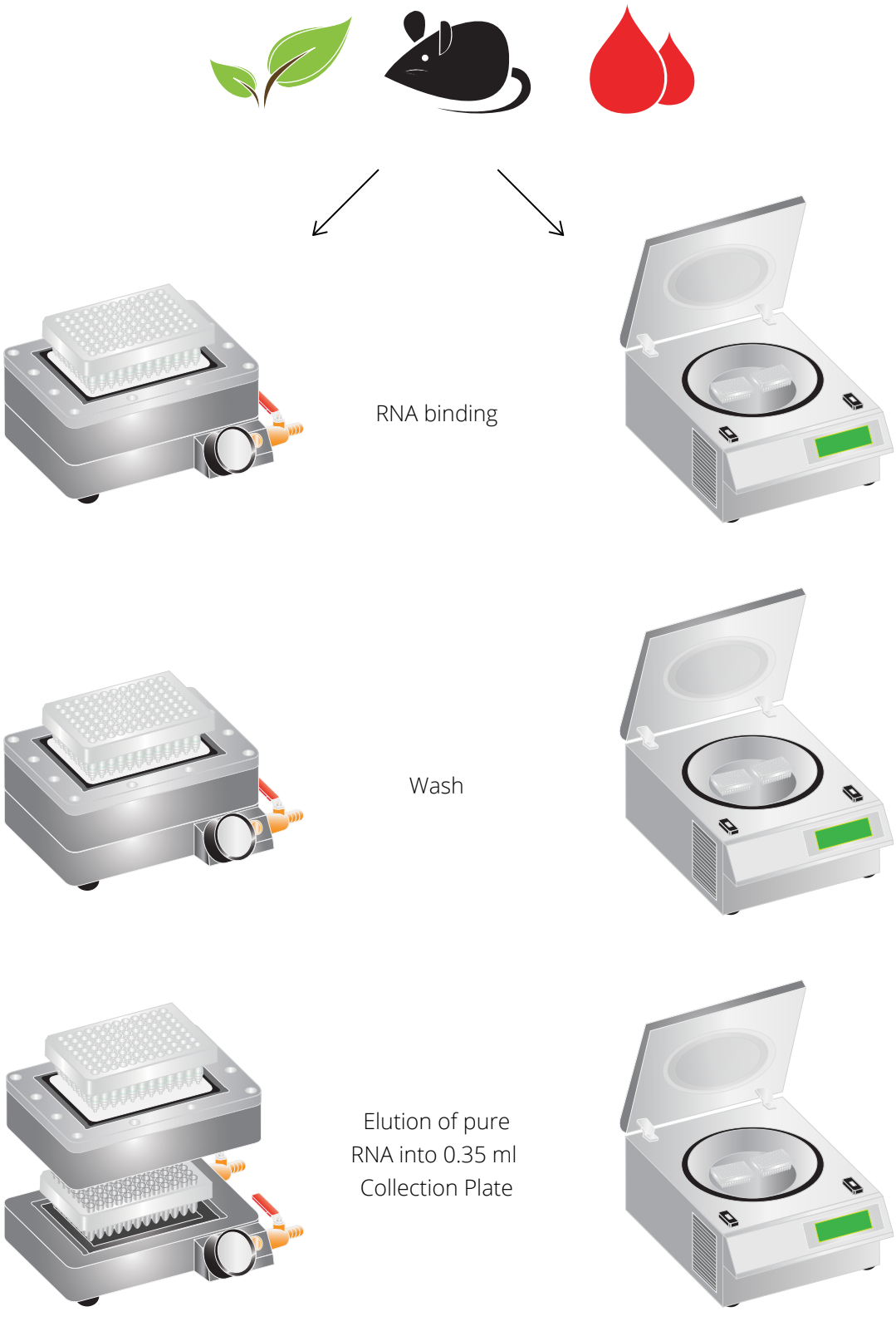
[4] 96 Deep Well Plates are reusable. After use, rinse the plate with water then incubate in 0.4M HCl for 1 minute at room temperature. Wash the plate thoroughly with ddH_2O. The plate can be autoclaved after being washed.

IMPORTANT BEFORE USE!

Isolate 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.

Quick Protocol Diagram

Transfer Isolate Reagent treated samples
to 96 Well RNA Binding plate



Isolate 96 Well RNA Kit Protocol

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

IMPORTANT BEFORE USE!

1. 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. 96 Deep Well Plates are reusable. After use, rinse the plate with water then incubate in 0.4M HCl for 1 minute at room temperature. Wash the plate thoroughly with ddH_2O. The plate can be autoclaved after being washed.

Additional Requirements:

Centrifuge with microplate buckets/vacuum manifold, additional 96 Deep Well Plate, absolute ethanol, steal or ceramic beads, TissueLyser or mixer mill for animal/plant tissue. Lysozyme and Bacteria Lysis Buffer for bacteria, (optional) RNase-Free DNase I Set can be purchased directly from IBI - See page 8.

Sample Homogenization and Lysis (Centrifuge and Vacuum)

Sample	Procedure
Adherent Cultured Cells	1. Remove the culture medium from the culture dish. 2. Directly add **500 µl of Isolate Reagent** to the culture dish **(up to 2 x 10^6 cells)** then lyse the cells directly in the culture dish by pipetting several times. 3. Incubate for 5 minutes at room temperature. 4. Transfer the sample to each tube of **Microtubes (Racked)**, being careful not to touch the rims with pipette tips
Suspension Cultures Cells	1.**Transfer cells (up to 2 x 10^6)** to each tube of **Microtubes (Racked)**, being careful not to touch the rims with pipette tips. 2. Seal the tubes with **Caps for Microtubes** then cover rack with the plastic cover. 3. Centrifuge at 300 x g for 5 minutes. 4. Remove caps then completely remove the culture medium. 5. Add **500 µl of Isolate Reagent** into each tube then re-suspend the cell pellet by pipette. 6. Incubate for 5 minutes at room temperature.
Animal and Plant Tissue	1. Add steal beads or ceramic beads (RNase-free) into each tube of **Microtubes (Racked).** 2. Transfer **10-20 mg of animal or plant tissue** and **500 µl of Isolate Reagent** into each tube, being careful not to touch the rims with pipette tips. 3. Seal the tubes with **Caps for Microtubes** then cover rack with the plastic cover. 4. Homogenize samples using a TissueLyser or mixer mill then incubate for 5 minutes at room temperature. 5. Centrifuge at 5,000-6,000 x g for 5 minutes to remove cell debris then transfer the clear supernatant to each tube of a new **Microtubes (Racked)**.

Body Fluids (blood, buffy coat, plasma, serum)	1. **Transfer up to 150 µl of liquid sample to each tube of Microtubes (Racked) then add 3 volumes of Isolate Reagent per 1 volume of sample (3:1)** and mix well by pipette, being careful not to touch the rims with pipette tips. 2. Seal the tubes with **Caps for Microtubes** then cover rack with the plastic cover. 3. Incubate for 5 minutes at room temperature. 4. Centrifuge at 5,000-6,000 x g for 5 minutes to remove cell debris then transfer the clear supernatant to each tube of new **Microtubes (Racked)**.
Bacteria	1. **Transfer bacterial cells (up to 1 x 10^9) to each tube of Microtubes (Racked)**, being careful not to touch the rims with pipette tips. 2. Seal the tubes with **Caps for Microtubes** then cover rack with the plastic cover. 3. Centrifuge at 5,000-6,000 x g for 3 minutes. 4. Remove caps then completely remove the culture medium. 5. **Transfer 100 mg of Lysozyme powder** to a 15 ml centrifuge tube (RNase-free) containing **10 ml of Bacteria Lysis Buffer** then vortex until the **Lysozyme** powder is completely dissolved. (See page 8 for product model numbers) 6. Add **100 µl of Bacteria Lysis Buffer** containing Lysozyme into each tube then re-suspend the cell pellet by pipette. 7. Incubate for 5 minutes at room temperature. 8. Add **500 µl of Isolate Reagent** into each tube then mix well by pipette. 9. Incubate for 5 minutes at room temperature.

Centrifuge Protocol Procedure

2. RNA Binding

Add 1 volume of absolute ethanol directly to 1 volume of sample mixture (1:1) into each tube of **Microtubes (Racked)**, being careful not to touch the rims with pipette tips. Seal the tubes with new caps, and cover the rack with the plastic cover. Mix by shaking vigorously for 15-30 seconds. Centrifuge at 1,000 x g for 30 seconds to collect any lysate from the caps. Place the **96 Well RNA Binding Plate on a 96 Deep Well Plate**. Remove caps then transfer **500 µl of sample lysate** into each well of the **96 Well RNA Binding Plate**, being careful not to get any lysate on the the rims of the wells. Centrifuge the **96 Well RNA Binding Plate** and **96 Deep Well Plate** together at 3,000 x g for 5 minutes. Transfer the remaining sample lysate into each well of **96 Well RNA Binding Plate**, being careful not to get any lysate on the the rims of the wells. Centrifuge the **96 Well RNA Binding Plate** and **96 Deep Well Plate** together at 3,000 x g for 5 minutes. Discard the flow-through then place the **96 Well RNA Binding Plate** back on the **96 Deep Well Plate**.

Optional In-Column DNase I Treatment

Add 400 µl of Wash Buffer (make sure ethanol was added) into each well of the 96 Well RNA Binding Plate, being careful not to get any buffer on the the rims of the wells. Centrifuge the 96 Well RNA Binding Plate and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through. Place the 96 Well RNA Binding Plate back on the 96 Deep Well Plate. Prepare DNase I solution in a 15 ml centrifuge tube (RNase-free) as follows:

DNase I (2U/µl)	0.5 ml
DNase I Reaction Buffer	4.5 ml
Total Volume	5 ml

See page 8 for product model numbers

Gently pipette the DNase I solution to mix (DO NOT vortex) then add 50 µl of DNase I solution into the CENTER of each well of the 96 Well RNA Binding Plate. Incubate for 15 minutes at room temperature (20-30°C) then proceed with RNA Wash.

3. Wash

Add 400 µl of Pre-Wash Buffer (make sure ethanol was added) to each well of the **96 Well RNA Binding Plate** then centrifuge the **96 Well RNA Binding Plate** and **96 Deep Well Plate** together at 3,000 x g for 5 minutes. Discard the flow-through then place the **96 Well RNA Binding Plate** back on the **96 Deep Well Plate**. **Add 700 µl of Wash Buffer (make sure ethanol was added)** to each well of the **96 Well RNA Binding Plate** then centrifuge the **96 Well RNA Binding Plate** and **96 Deep Well Plate** together at 3,000 x g for 5 minutes. Discard the flow-through then place the **96 Well RNA Binding Plate** back on the **96 Deep Well Plate**. Wash the **96 Well RNA Binding Plate** again with **700 µl of Wash Buffer**. Discard the flow-through then place the **96 Well RNA Binding Plate** back on the **96 Deep Well Plate**. Centrifuge the **96 Well RNA Binding Plate** and **96 Deep Well Plate** together at 3,000 x g for 10 minutes to dry the membrane.

4. Elution

Remove the **96 Well RNA Binding Plate** from the **96 Deep Well Plate** then blot the nozzles on a clean absorbent paper towel to remove residual ethanol. Place the **96 Well RNA Binding Plate** on top of a **0.35 ml Collection Plate**. **Add 60-80 µl of RNase-free Water** into the CENTER of each well of the **96 Well RNA Binding Plate**. Let stand for at least 2 minutes to ensure the RNase-free water is absorbed by the membrane. Centrifuge the **96 Well RNA Binding Plate** and **0.35 ml Collection Plate** together at 3,000 x g for 5 minutes. Seal the **0.35 ml Collection Plate** with **Adhesive Film** and store the purified RNA at -70°C.

Vacuum Protocol Procedure

2. Vacuum Manifold Preparation

Place the **96 Deep Well Plate** on the manifold base. Place the binding top plate on the manifold base. Place the **96 Well RNA Binding Plate** on the binding top plate aperture. Attach the vacuum manifold to a vacuum source.

3. RNA Binding

Add 1 volume of absolute ethanol directly to 1 volume of sample mixture (1:1) into each tube of **Microtubes (Racked)**, being careful not to touch the rims with pipette tips. Seal the tubes with new caps, and cover the rack with the plastic cover. Mix by shaking vigorously for 15-30 seconds. Centrifuge at 1,000 x g for 30 seconds to collect any lysate from the caps. Remove caps then transfer sample lysate (approx. 1 ml) into each well of the **96 Well RNA Binding Plate**, being careful not to get any lysate on the the rims of the wells. Seal unused wells of the 96 Well RNA Binding Plate with **Adhesive Film**. Apply vacuum at 15 inches Hg until sample passes through the **96 Well RNA Binding Plate** then turn off the vacuum. Discard the flow-through and re-assemble manifold.

Optional In-Column DNase I Treatment

Add 400 µl of Wash Buffer (make sure ethanol was added) into each well of the 96 Well RNA Binding Plate, being careful not to get any buffer on the the rims of the wells. Apply vacuum at 15 inches Hg until sample passes through the 96 Well RNA Binding Plate then turn off the vacuum.

Prepare DNase I solution in a 15 ml centrifuge tube (RNase-free) as follows:

DNase I (2U/µl)	0.5 ml
DNase I Reaction Buffer	4.5 ml
Total Volume	5 ml

See page 8 for product model numbers

Gently pipette the DNase I solution to mix (DO NOT vortex) then add 50 µl of DNase I solution into the CENTER of each well of the 96 Well RNA Binding Plate. Incubate for 15 minutes at room temperature (20-30°C) then proceed with RNA Wash.

4. Wash

Add 400 µl of Pre-Wash Buffer (make sure ethanol was added) to each well of the **96 Well RNA Binding Plate**. Apply vacuum at 15 inches Hg until **Pre-Wash Buffer** passes through the 96 Well RNA Binding Plate (approx. 10 seconds). Turn off the vacuum. Discard the flow-through and re-assemble the manifold.

Add 1 ml of Wash Buffer (make sure ethanol was added) to each well of the **96 Well RNA Binding Plate**. Apply vacuum at 15 inches Hg until **Wash Buffer** passes through the **96 Well RNA Binding Plate** (approx. 10 seconds). Turn off the vacuum. Discard the flow-through and re-assemble the manifold. Wash the **96 Well RNA Binding Plate** again with **1 ml of Wash Buffer**. Apply vacuum for an additional 10 minutes to dry the membrane then turn off the vacuum.

5. Elution

Remove the **96 Well RNA Binding Plate** from the binding top plate aperture then blot the nozzles on a clean absorbent paper towel to remove residual ethanol. Remove the **96 Deep Well Plate** from the manifold base then place the collection plate spacer on the manifold base. Place a **0.35 ml collection Plate** on top of the collection plate spacer. Place the binding top plate back on the manifold base then place the **96 Well RNA Binding Plate** back on the binding top plate aperture. **Add 60-80 µl of RNase-free Water** into the CENTER of each well of the **96 Well RNA Binding Plate**. Let stand for at least 2 minutes to ensure the **RNase-free Water** is absorbed by the membrane. Apply vacuum at 15 inches Hg for 5 minutes then turn off the vacuum. Seal the **0.35 ml Collection Plate** with **Adhesive Film** and store the purified RNA at -70°C.

Troubleshooting

Low Yield

Sample lysis or homogenization was incomplete.
Starting material should be reduced and completely dissolved in Isolate Reagent.

Incorrect RNA elution.
Make sure RNase-free Water is added to the center of each well of the 96 Well RNA Binding Plate and is absorbed completely.

Precipitates may form during the RNA binding step after adding 1 volume of absolute ethanol to the sample mixture in Isolate Reagent if too much sample material is used.
Reduce sample to half of the original amount.

Degraded RNA

Incorrect sample preparation and/or storage.
Process or freeze samples immediately after collection.

Incorrect storage temperature.
Extracted RNA should be stored at -70°C.

Low RNA A260/A280

Volume of Isolate Reagent was insufficient for proper sample homogenization.
Volume of Isolate Reagent is sample dependent and should be added according to the sample homogenization specifications.

Incomplete wash step.
Wash the 96 Well RNA Binding Plate with appropriate volume of ethanol added Wash Buffer.

Eluted RNA does not perform well in downstream applications

Residual ethanol contamination.
Following the wash step, dry the 96 Well RNA Binding Plate with additional centrifugation at 3,000 x g or with additional vacuum for 10 minutes to ensure the membrane is completely dry.

Isolate 96 Well RNA Pure Kit Functional Test Data (HeLa Cells)

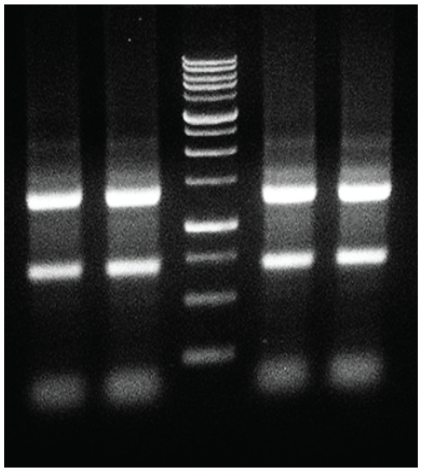
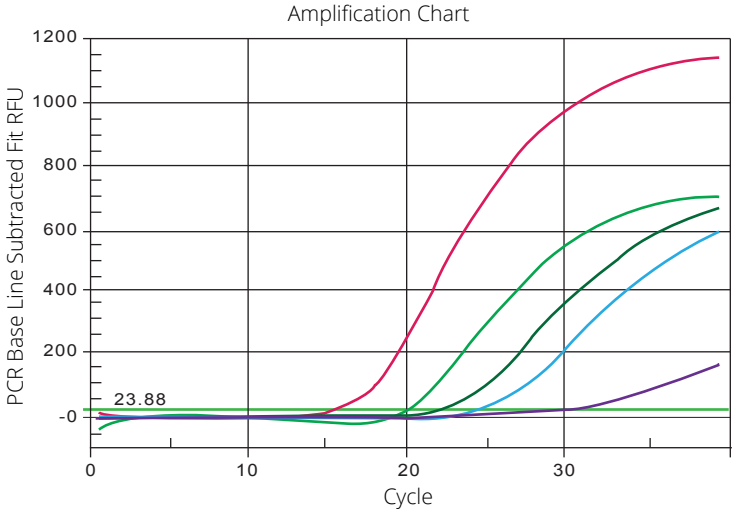


Figure 1.
RNA was purified using the Isolate 96 Well RNA Pure Kit in parallel to the similar product from competitor Z. 5 x 10^5 HeLa cells were homogenized using Isolate Reagent and competitor Z tri reagent. RNA was then purified using the corresponding kits binding plate procedure. 10 µl from a 50 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	8.1µg
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

Isolate 96 Well RNA Pure Kit Real-Time PCR Data



RNA was extracted from 200 µl of whole human blood (5.2 x 10^{10} copy/ml) using 96 Well TriRNA Pure Kit.

RNA standard (10^7 copy/ml)
RNA standard (10^6 copy/ml)
RNA standard (10^5 copy/ml)

RNA standard (10^4 copy/ml)

Figure 2.
Quantitative analysis of human beta globin mRNA extracted by 96 Well RNA 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.

Optional/Additional Requirements

RNase-Free DNase I set	Lysozyme & Bacteria Lysis Buffer
IB47450 – 275 µl	IB47633 – 110 mg
IB47451 – 550 µl	IB47634 – 250 mg
IB47452 – 1.65 ml	

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