IBI Isolate

Store at 2ºC to 25ºC
For research use only

Catalogue Numbers	Quantity
IB47600	4 ml
IB47601	100 ml
IB47602	200 ml

Introduction

IBI Isolate is a phenol, chloroform and guanidine isothiocyanate based scalable solution for extracting high-quality total RNA as well as simultaneous extraction of RNA, DNA and protein from a wide variety of samples such as blood, buffy coat, plasma, serum, cultured cells and tissue. The extracted RNA can be used directly in a variety of downstream applications such as cDNA Library Construction, Cloning, RT-PCR (Endpoint), Real-Time PCR, Nuclease Protection Assays and Northern Blotting.

Quality Control

IBI Isolate is tested on a lot-to-lot basis. RNA from a 1 ml human blood sample is extracted using IBI Isolate. 10 µl from a 50 µl eluate of RNA is analyzed by electrophoresis on a 0.8% agarose gel.

Advantages

- Extract total RNA or simultaneous RNA, DNA and protein within 1 hour
- Sample: up to 300 µl (blood, buffy coat, serum, plasma), up to 5 x 10^6 (cultured cells), 50-100 mg (tissue)
- Scalable
- Format: phenol, chloroform and guanidine isothiocyanate

Applications

cDNA Library Construction, Cloning, RT-PCR (Endpoint), Real-Time PCR, Nuclease Protection Assays and 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.

Additional Requirements

RNA Extraction: chloroform, isopropanol, 70% ethanol, RNase-free Water, 1.5 ml microcentrifuge tubes (RNase-free)
DNA Extraction: chloroform, absolute ethanol, 70% ethanol, sodium citrate/ethanol solution (0.1 M sodium citrate in 10% ethanol, pH8.5), 8 mM NaOH solution or TE Buffer pH8.5, 1.5 ml microcentrifuge tubes
Protein Extraction: chloroform, absolute ethanol, 95% ethanol, wash solution (0.3 M guanidine hydrochloride in 95% ethanol), 1% SDS, 15 ml centrifuge tube, 1.5 ml microcentrifuge tubes

Components and Storage

IBI Isolate is shipped at room temperature and can be stored dry at 2ºC to 25ºC for up to 9 months.

IBI Isolate Real-Time PCR Data

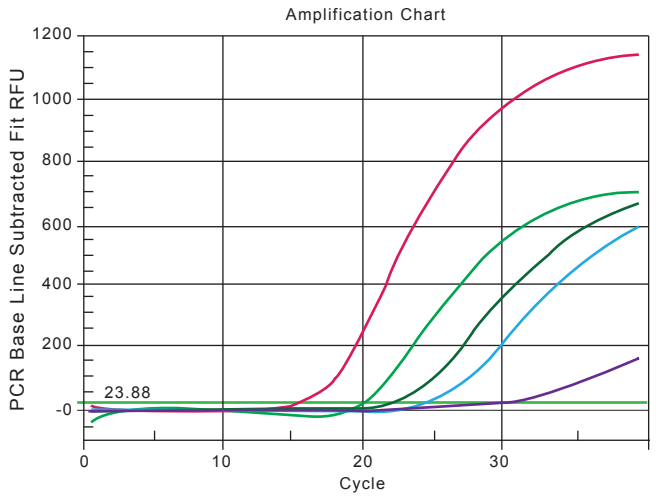

Figure 1. Quantitative analysis of human beta globin mRNA extracted by IBI Isolate using a Taqman probe 1-step qRT-PCR assay. The assay was run on a BioRad IQ5 thermal cycler. The high yield, high quality extracted RNA was amplified quickly following a very short C_T (threshold cycle) compared to the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

RNA Extraction Protocol Procedure

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

1. Sample Homogenization

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 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 new 1.5 ml of 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 cells by centrifugation at 300 x g for 5 minutes to form a cell pellet. 3. Remove the culture medium completely. 4. **Add 1 ml of IBI Isolate** to the cell pellet and lyse the cells by pipetting several times. 5. Incubate the sample mixture for 5 minutes at room temperature.
Tissue	1. **Add 1 ml of IBI Isolate to 50–100 mg of tissue** sample. 2. Homogenize tissue samples using a glass-Teflon or Polytron homogenizer. 3. Incubate the homogenized sample for 5 minutes at room temperature. 4. Transfer the sample to a new 1.5 ml of microcentrifuge tube (RNase-free).
Body Fluids (blood, buffy coat, plasma, serum)	1. **Transfer up to 300 µl of liquid sample** to a 1.5 ml of microcentrifuge tube (RNase-free). 2. **Add 3 volumes of IBI Isolate to each volume of liquid sample (3:1)** 3. Mix well by vortex. 4. Incubate the sample mixture for 5 minutes at room temperature.
NOTE: For samples which contain high levels of fat, proteins, polysaccharides, or extracellular material, perform this optional step following sample homogenization. However, if DNA extraction is required, DO NOT perform this additional step. 1. Centrifuge the sample at 12-16,000 x g for 10 minutes to remove insoluble particles. NOTE: Following centrifugation of high fat content samples, a layer a fat will float on the supernatant. Remove and discard the fatty layer. 2. Transfer the clear supernatant to a new 1.5 ml microcentrifuge tube (RNase-free). 3. Proceed to Step 2 Phase Separation.	

2. Phase Separation

1. **Add 200 µl of chloroform to the sample per 1 ml of IBI Isolate used in sample homogenization**.
2. Shake the microcentrifuge tube vigorously for 10 seconds.
3. Centrifuge the sample at 12–16,000 x g for 15 minutes at 4ºC to separate the phases.

NOTE: RNA is in the colorless upper aqueous phase which is approximately 50% of the total volume.

4. Transfer the upper aqueous phase to a new 1.5 ml microcentrifuge tube (RNase-free).

NOTE: Be careful not to draw any of the interphase layer (white) or organic phase layer (red) when transferring the aqueous layer. If DNA isolation is required, save the interphase and organic phase then proceed with the DNA Extraction protocol on page 3.

3. RNA Precipitation

1. **Add 1 volume of isopropanol to the aqueous phase** then mix by inverting the tube several times.
2. Incubate the sample mixture for 10 minutes at room temperature.
3. Centrifuge the sample at 12–16,000 x g for 10 minutes at 4ºC to form a tight RNA pellet.
4. Carefully remove and discard the supernatant.

4. RNA Wash

1. **Add 1 ml of 70% ethanol** to wash the RNA pellet then vortex briefly.
2. Centrifuge the sample at 12–16,000 x g for 5 minutes at 4ºC.
3. Being careful not to contact the RNA pellet, remove the supernatant with a pipette.
4. Air-dry the RNA pellet for 5-10 minutes at room temperature.

NOTE: DO NOT dry the RNA pellet by vacuum centrifuge and avoid over drying the RNA pellet.

5. RNA Resuspension

1. **Add 20-50 µl of RNase-free Water** to resuspend the RNA pellet.
2. Incubate at 55-60ºC for 10-15 minutes to dissolve the RNA pellet.

NOTE: Occasionaly tapping the bottom of the tube during incubation will promote RNA rehydration.

The RNA is ready for downstream applications or storage at -70ºC.

DNA Extraction Protocol Procedure

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

NOTE: DNA is extracted from the interphase and organic phase which was saved following the removal of the aqueous phase in the Phase Separation step.

1. DNA Precipitation

1. Carefully remove any residual upper aqueous phase layer.

NOTE: This step is critical for ensuring extracted DNA quality.

2. **Add 300 µl of absolute ethanol to the sample per 1 ml of IBI Isolate used in sample homogenization**.
3. Mix by inverting the tube several times.
4. Incubate the sample for 5 minutes at room temperature.
5. Centrifuge the sample at 2,000 x g for 5 minutes at 4ºC then carefully remove the supernatant.

NOTE: If protein isolation is required, save the phenol-ethanol supernatant in a 15 ml centrifuge tube. The supernatant can be stored at -70ºC for several months.

2. DNA Wash

1. **Add 1 ml of sodium citrate/ethanol solution (0.1 M sodium citrate in 10% ethanol, pH8.5) to the sample per 1 ml of IBI Isolate used in initial sample homogenization**.
2. Incubate the sample for 30 minutes at room temperature. During incubation, gently invert the tube occasionally.
3. Centrifuge the sample at 2,000 x g for 5 minutes at 4ºC then remove the supernatant.
4. Repeat the above wash steps once.
5. **Add 1.5 ml of 70% ethanol to the sample per 1 ml of IBI Isolate used in the initial sample homogenization**.
6. Incubate for 10-20 minutes at room temperature. During incubation, gently invert the tube occasionally.
7. Centrifuge the sample at 2,000 x g for 5 minutes at 4ºC then carefully remove the supernatant.
8. Air-dry the DNA pellet for 5-10 minutes at room temperature.

NOTE: DO NOT dry the DNA pellet by vacuum centrifuge and avoid over drying the DNA pellet.

3. DNA Resuspension

1. **Add 300 µl of 8 mM NaOH solution or TE Buffer pH8.5** to the DNA pellet.

NOTE: Resuspending the DNA pellet in a weak base solution is recommended.

2. Incubate the DNA sample at 55-60ºC for 10-15 minutes to dissolve the DNA pellet.

NOTE: Occasionaly tapping the bottom of the tube during incubation will promote DNA rehydration.

3. Centrifuge the sample at 12-16,000 x g for 10 minutes to remove the insoluble particles.
4. Transfer the supernatant containing the DNA to a new 1.5 ml microcentrifuge tube.

The DNA is ready for downstream applications or storage at -20ºC.

Protein Extraction Protocol Procedure

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

NOTE: Protein is extracted from the phenol-ethanol supernatant which was saved from the DNA Extraction Protocol Procedure.

1. Protein Precipitation

1. **Add 1.5 ml of isopropanol to the phenol-ethanol supernatant per 1 ml of IBI Isolate** used in sample homogenization then mix by inverting the tube several times.
2. Incubate the sample for 10 minutes at room temperature.
3. Centrifuge the sample at 12,000 x g for 10 minutes at 4ºC to precipitate the protein then carefully remove the supernatant.

2. Protein Wash

1. **Add 2 ml of wash solution (0.3 M guanidine hydrochloride in 95% ethanol) to the protein pellet per 1 ml of IBI Isolate** used in the initial sample homogenization.
2. Incubate for 20 minutes at room temperature. The protein sample could be stored in the wash solution for one year at -20ºC.
3. Centrifuge the sample at 7,500 x g for 5 minutes at 4ºC then remove the supernatant.
4. Repeat above wash steps two more times.
5. **Add 2 ml of 100% ethanol to the protein pellet** after the third wash then vortex.
6. Incubate for 20 minutes at room temperature.
7. Centrifuge the sample at 7,500 x g for 5 minutes at 4ºC then carefully remove the supernatant.
8. Air-dry the protein pellet for 5-10 minutes at room temperature.

NOTE: DO NOT dry the protein pellet by vacuum centrifuge and avoid over drying the protein pellet.

3. Protein Resuspension

1. **Add 200 µl of 1% SDS to the protein pellet** then resuspend the pellet by pipetting.
2. Incubate the sample at 50ºC for 5-10 minutes to completely dissolve the protein pellet.
3. Centrifuge at 10,000 xg for 10 minutes at 4ºC to remove the insoluble particles.
4. Transfer the supernatant containing the protein to a new 1.5 ml microcentrifuge tube.

The protein is ready for downstream applications or storage at -20ºC.

Troubleshooting

Problem	Cause	Solution
Low Yield	A. Sample lysis or homogenization was incomplete B. DNA/RNA/Protein pellet was not dissolved completely	A. Starting material should be reduced and completely dissolved in IBI Isolate. B. Increase incubation temperature to 60ºC and increase incubation time to 15 minutes. If the pellet is still not dissolved, pipette until it dissolves completely.
Degraded DNA/RNA/Protein	A. Incorrect sample preparation and/or final storage B. Incorrect sample storage temperature	A. Process or freeze samples immediately after collection. B. Extracted RNA should be stored at -70ºC. Extracted DNA and Protein should be stored at -20ºC.
RNA/DNA Contamination	A. When removing the aqueous phase, the interephase and/or organic phase were drawn into the pipette B. The aqueous phase was not removed completely C. The DNA pellet was not washed completely	A. Leave a small amount of aqueous phase to avoid drawing the interphase and/or organic phase into the pipette. B. Remove the remaining aqueous phase prior to DNA extraction. C. The DNA pellet should be washed with 0.1 M sodium citrate in 10% ethanol pH8.5.
Low RNA A260/A280	A. Volume of IBI Isolate was insufficient for proper sample homogenization	A. Volume of IBI Isolate is sample dependent and should be added according to the sample homogenization specifications.
Low DNA A260/A280	A. Residual phenol contamination	A. The DNA pellet can be washed an additional time with 0.1 M sodium citrate in 10% ethanol pH8.5.

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 Maxi Kit (Blood/Cultured Cell)	10 preps	IB47330
Total RNA Mini Kit (Tissue)	50/100 preps	IB47301/302
Total RNA Maxi Kit (Tissue)	10 preps	IB47310
Total RNA Mini Kit (Plant)	50/100 preps	IB47341/342
Total RNA Maxi Kit (Plant)	10 preps	IB47350
rBAC Mini RNA Bacteria Kit	100/300 preps	IB47421/412
rYeast Total RNA Mini Kit	50/100/300 preps	IB47411/422
96-Well Total RNA Extraction Kit (Plant)	4/10 x 96 preps	IB47381/382
96-Well Total RNA Extraction Kit	4/10 x 96 preps	IB47360/361
miRNA Isolation Kit	100 preps	IB47371
IBI Isolate	100/200 rxns	IB47601/602
IBI Tri-Isolate	100/200 rxns	IB47631/632
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!