For Research Use Only

IBI SCIENTIFIC

INSTRUCTION MANUAL

IBI Plant Isolate

IB47610 (4 Prep Sample Kit)
IB47611 (100 Prep Kit)
IB47612 (200 Prep Kit)

Catalogue Numbers	Quantity
IB47610	4 ml
IB47611	100 ml
IB47612	200 ml

Introduction

IBI Plant Isolate provides a quick and easy 3 step CTAB and chloroform based method to isolate total DNA (including genomic, mitochondrial and chloroplast DNA) from a variety of plant species (including algae and cyanobacteria). This unique reagent is able to lyse most common plant samples and plant samples with high a polysaccharide content. The extracted DNA is suitable for routine PCR screening, Real-Time PCR, Southern Blotting, Mapping and RFLP. Phenol extraction is not required and the entire procedure can be completed within 50 minutes.

Quality Control

IBI Plant Isolate is tested on a lot-to-lot. 50 mg of fresh Arabidopsis leaves are initially ground in IBI Plant Isolate. A 15 µl aliquot of extracted genomic DNA from a 100 µl eluate is analyzed by electrophoresis on a 1% agarose gel.

Advantages

- High molecular weight genomic DNA extraction from a variety of plant species
- Sample: up to 1 g of fresh plant tissue and up to 0.5 g of dry plant tissue
- Scalable, simple and gentle CTAB and chloroform based DNA precipitation method
- Cost effective

Applications

PCR, Real-Time PCR, Southern Blotting, Mapping and RFLP

Caution

IBI Plant Isolate contains irritants. During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.

Additional Requirements

Mortar and pestle, 1.5 ml microcentrifuge tubes or 15 ml centrifuge tubes, absolute ethanol for preparing 70% ethanol in water, chloroform, isopropanol, TE buffer or ddH2O.

Components and Storage

Item	Volume	Product	Shipping	Storage
IBI Plant Isolate	4 ml	IB47610	room temperature	dry at room temperature (15-25ºC) 100 ml IB47611 for up to 1 year
	100 ml	IB47611		
	200 ml	IB47612		
RNase A (50 mg/ml)	N/A	IB47610	room temperature	4ºC for extended periods
	50 µl	IB47611		
	100 µl	IB47612		

IBI Plant Isolate Functional Test Data

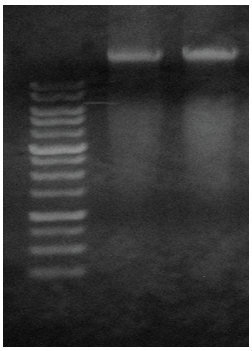

Figure 1. Genomic DNA (approximately 30 kb) was extracted using IBI Plant Isolate. 50 mg of fresh Arabidopsis leaves were initially ground in IBI Plant Isolate. A 15 µl aliquot of extracted genomic DNA from a 50 µl eluate was analyzed by electrophoresis on a 1% agarose gel.

M = 1 Kb DNA Ladder

Test	DNA Concentration	260/280	260/230	Yield
1	380.7 µg/ml	1.97	1.88	19.04 µg
2	316.9 µg/ml	1.96	1.89	15.85 µg

Fast PCR Grade DNA Protocol Procedure

Please read the entire instruction manual prior to starting the Protocol Procedure.
Use this protocol procedure for purifying PCR grade DNA for routine PCR assays.

1. Plant Tissue Homogenization

1. Cut off **50 mg of fresh plant tissue or 25 mg of dry plant tissue**.
2. Freeze the sample with liquid nitrogen (some plant samples can be disrupted without liquid nitrogen).
3. Grind the sample to a fine powder using a mortar and pestle.

2. Lysis

1. Add **800 µl of IBI Plant Isolate and 0.5 µl of RNase A** to the sample in the mortar.
2. Continue grinding the sample until it is completely dissolved.
3. Transfer the sample lysate to a 1.5 ml microcentrifuge tube.
4. Incubate the sample lysate at 65ºC for 15 minutes then centrifuge at 14-16,000 x g for 3 minutes.
5. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

3. DNA Precipitation

1. Add **600 µl of isopropanol** to the supernatant in the 1.5 ml microcentrifuge tube.
2. Mix the sample by gently inverting 20 times then let stand for 5 minutes at room temperature.
3. Centrifuge at 14-16,000 x g for 15 minutes to form a tight, well formed DNA pellet.
4. Carefully remove the supernatant then add **1 ml of 70% ethanol** to the DNA pellet and wash by gently inverting 20 times.
5. Centrifuge at 14-16,000 x g for 3 minutes.
6. Carefully remove the supernatant then air-dry the DNA pellet for 10-15 minutes at room temperature.

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

7. Add **50-100 µl of TE buffer or ddH2O** to the DNA pellet then incubate at 65°C for 5 minutes to dissolve the DNA.

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

8. Centrifuge at 14-16,000 x g for 1 minute then transfer the supernatant (containing the purified DNA) to a clean 1.5 ml microcentrifuge tube. The purified DNA is ready for routine PCR assays.

High Purity and High Yield DNA Protocol Procedure

Please read the entire instruction manual prior to starting the Protocol Procedure.
Use this protocol procedure for purifying high purity and high yield DNA.

1. Plant Tissue Homogenization

1. Cut off **100 mg of fresh plant tissue or 50 mg of dry plant tissue**.
2. Freeze the sample with liquid nitrogen (some plant samples can be disrupted without liquid nitrogen).
3. Grind the sample to a fine powder using a mortar and pestle.

2. Lysis

1. Add **1 ml of IBI Plant Isolate and 0.5 µl of RNase A** to the sample in the mortar.

NOTE! If using more than 100 mg of plant tissue, scale IBI Plant Isolate proportionately (see table on page 3).

2. Continue grinding the sample until it is completely dissolved then transfer the sample lysate to a 1.5 ml microcentrifuge tube.

NOTE! If using more than 100 mg of plant tissue, transfer the sample lysate to a 15 ml centrifuge tube.

4. Incubate the sample lysate at 65°C for 30 minutes then centrifuge at 14-16,000 x g for 5 minutes.
5. Transfer the supernatant to a new 1.5 ml microcentrifuge tube or a new 15 ml centrifuge tube for larger sample sizes.

3. DNA Extraction

Standard Samples:

1. Add **600 µl of chloroform** to the supernatant.

NOTE! Scale the chloroform proportionately if using larger sample sizes (see table on page 3).

2. Shake the tube vigorously then centrifuge at 14-16,000 x g for 5 minutes.
3. Carefully remove the upper layer and transfer it to a new 1.5 ml microcentrifuge tube or a new 15 ml centrifuge tube for larger sample sizes.

High Polysaccharide Samples:

1. Add a **1/10 volume of IBI Plant Isolate and 600 µl of chloroform** to the supernatant from Step 2.

NOTE! Scale IBI Plant Isolate and chloroform proportionately if using larger sample sizes (see table on page 3).

2. Shake the tube vigorously then centrifuge at 14-16,000 x g for 5 minutes.
3. Carefully remove the upper layer and transfer it to a new 1.5 ml microcentrifuge tube or a new 15 ml centrifuge tube for larger sample sizes.

4. DNA Precipitation

1. Add 800 µl of isopropanol to the 1.5 ml microcentrifuge tube containing the upper layer from step 3.

NOTE! Scale isopropanol proportionately if using larger sample sizes (see table below).

2. Mix the sample by gently inverting 20 times then let stand for 5 minutes at room temperature.

NOTE! DNA precipitation can be increased with extended standing time.

3. Centrifuge at 14-16,000 x g for 20 minutes to form a tight, well formed DNA pellet.
4. Carefully remove the supernatant then add **1 ml of 70%** ethanol to the DNA pellet and wash by gently inverting 20 times.
5. Centrifuge at 14-16,000 x g for 3 minutes.
6. Carefully remove the supernatant then air-dry the DNA pellet for 10-15 minutes at room temperature.

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

7. Add 50-100 µl of TE buffer or ddH2O to the DNA pellet then incubate at 65ºC for 10 minutes to dissolve the DNA.

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

8. Centrifuge at 14-16,000 x g for 1 minute then transfer the supernatant (containing the purified DNA) to a clean 1.5 ml microcentrifuge tube. The purified DNA is ready for routine PCR assays.

Scaling Large Sample Volumes

Plant tissue	100 mg	500 mg
Tube size	1.5 ml	15 ml
IBI Plant Isolate	1 ml	5 ml
RNase A (50 mg/ml)	0.5 µl	2.5 µl
Chloroform	600 µl	3 ml
Isopropanol	800 µl	4 ml
70% ethanol	1 ml	5 ml

Troubleshooting

Problem	Volume	Shipping
Low Yield	**A**. Sample lysis or homogenization was incomplete is completely evaporated. **B**. Incorrect DNA precipitation	**A**. Starting material should be reduced and completely dissolved in IBI Plant Isolate. Increase incubation time to 1 hour during lysis. **B**. Following isopropanol addition, increase standing time to improve DNA precipitation. Following centrifugation, carefully remove the supernatant without contacting the DNA pellet.
Slow Rehydration	**A**. The DNA pellet is too dry	**A**. Increase incubation time and tap the bottom of the tube occasionally to facilitate rehydration.
Eluted DNA does not perform well in downstream applications	**A**. Residual ethanol contamination	**A**. Increase DNA pellet drying time to ensure residual ethanol is completely evaporated.

Related DNA/RNA 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
Total RNA Mini Kit (Blood/Cultured Cell)	50/100/300 preps	IB47321/322/323
Genomic DNA Extraction and Purification		
Product	Package size	Catalogue number
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	IB47201/202
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10 preps	IB47210
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
Genomic DNA Maxi Kit (Plant)	10/25 preps	IB47240/241
gBAC Mini DNA Bacteria Kit	100/300 preps	IB47291/292
gYEAST Genomic DNA Kit	100/300 preps	IB47266/267
96-Well Genomic DNA Extraction Kit	4/10 x 96 preps	IB47251/252
96-Well Genomic DNA Extraction Kit (Plant)	4/10 x 96 preps	IB47271/272
IBI Plant Isolate	100/200 rxns	IB47611/612
Plasmid DNA Purification		
Product	Package size	Catalogue number
I-Blue Mini Plasmid Kit	100/300 preps	IB47171/172
I-Blue Midi Plasmid Kit	25 preps	IB47181
I-Blue Midi Plasmid Kit (Endotoxin Free)	25 preps	IB47191
Fast Ion Plasmid Midi Kit	25 preps	IB47111
Fast Ion Plasmid Midi Kit (Endotoxin Free)	25 preps	IB47113
Fast Ion Plasmid Maxi Kit	10/25 preps	IB47121/122
Fast Ion Plasmid Maxi Kit (Endotoxin Free)	10/25 preps	IB47124/125
96-Well Plasmid Kit	4/10 x 96 preps	IB47151/152

Post Reaction DNA Purification		
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 Gel/PCR DNA Extraction Kit	4/10 x 96 preps	IB47040/050

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

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