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IBI SCIENTIFIC

INSTRUCTION MANUAL

gPURE Cell DNA Isolation Kit

IB47430, IB47431, IB47432

Advantages

Sample: Cultured cells

Yield: High yield, High quality DNA (A260/A280 = 1.8-2.0)

Format: Scalable DNA precipitation method Kit

Storage: Dry at room temperature (15-25°C) for up to 2 years, RNase A should be stored at 4°C for extended periods

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Introduction

The gPURE Cell DNA Isolation Kit offers a simple and gentle reagent DNA precipitation method for isolating high molecular weight genomic, mitochondrial or viral DNA suitable for sensitive downstream applications. This highly versatile solution based system can be scaled proportionately in order to satisfy larger sample volumes providing a convenient sample-storage procedure with minimal hands on time. Initially cells are lysed in the presence of detergents and a proprietary DNA stabilization solution followed by RNase A treatment. Once proteins and other contaminants are removed DNA is precipitated then rehydrated. The high quality extracted DNA is ready for use in a variety of downstream applications.

Quality Control

gPURE Cell DNA Isolation Kits are tested on a lot-to-lot basis by isolating DNA from (3-5 x 10^6) cultured cells. The isolated DNA (A260/A280 ratio of 1.8–2.0) is quantified with a spectrophotometer and analyzed by electrophoresis.

Components and Storage

Cell Lysis Buffer, Protein Removal Buffer, DNA Hydration Buffer should be stored dry at room temperature (15-25ºC) for up to 1 year. RNase A should be stored at 4ºC for extended periods.

gPURE Cell Kit	(3 ml)	(100 ml)	(1000 ml)
Catalogue No.	IB47430	IB47431	IB47432
Number of cells processed per kit	2 x 10^7	6 x 10^8	6 x 10^9 ml
Cell Lysis Buffer	3 ml	100 ml	1000 ml
Protein Removal Buffer	1 ml	40 ml	400 ml
DNA Hydration Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)	1 ml	50 ml	500 ml
RNase A (10 mg/ml)	25 µl	550 µl	5 ml

Scaling Large Sample Volumes

Cell number	0.5-1 x 10^6	3-5 x 10^6	3-5 x 10^7
Tube size	1.5 ml	1.5 ml	15 ml
Cell Lysis Buffer	150 µl	600 µl	6 ml
RNase A (10 mg/ml)	1 µl	3 µl	30 µl
Protein Removal Buffer	50 µl	200 µl	2 ml
Isopropanol	150 µl	600 µl	6 ml
70% ethanol	150 µl	600 µl	6 ml
DNA Hydration Buffer	50 µl	100 µl	200 µl

3-5 x 10^6 Cultured Cell Protocol Procedure

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

1. Sample Preparation

Adherent Cultured Animal Cells (trypsinize cells prior to harvesting)

Remove the culture medium and wash cells in PBS. Aspirate PBS and add 0.10-0.25% Trypsin in PBS. Once cells detach add the medium then transfer to a 1.5 ml microcentrifuge tube. Proceed with Suspension Cultured Animal cells.

Suspension Cultured Animal Cells

Transfer cells (3-5 x 10^6) to a 1.5 ml microcentrifuge tube then centrifuge for 5 minutes at 300 x g. Discard the supernatant retaining approximately 50 µl of residual buffer and cell pellet. Vortex the tube until the cell pellet is completely resuspended in the residual buffer.

2. Lysis

Add 600 µl of Cell Lysis Buffer to the tube then mix by vortex. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear/homogenous. During incubation, invert the tube every 3 minutes.

Optional RNA Removal Step

Following 60ºC incubation, add 3 µl of RNase A (10 mg/ml) to the sample then mix by vortex. Incubate at room temperature for 5 minutes.

3. Protein Removal

Add 200 µl of Protein Removal Buffer then vortex immediately for 10 seconds. Centrifuge at 14-16,000 x g for 3 minutes to form a tight pellet.

NOTE! If the pellet is loose then incubate on ice for 5 minutes followed by centrifugation at 14-16,000 x g for another 3 minutes.

4. DNA Precipitation

Transfer the supernatant to a new 1.5 ml microcentrifuge tube then add 600 µl of isopropanol and mix well by gently inverting 20 times. Centrifuge at 14-16,000 x g for 5 minutes then carefully discard the supernatant and add 600 µl of 70% ethanol to wash the pellet. Centrifuge at 14-16,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.

5. DNA Rehydration

Add 100 µl of DNA Hydration Buffer then gently vortex for 10 seconds. Incubate at 60°C for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

3-5 x 10^7 Cultured Cell Protocol Procedure

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

1. Sample Preparation

Adherent Cultured Animal Cells (trypsinize cells prior to harvesting)

Remove the culture medium and wash cells in PBS. Aspirate PBS and add 0.10-0.25% Trypsin in PBS. Once cells detach add the medium then transfer to a 15 ml centrifuge tube. Proceed with Suspension Cultured Animal cells.

Suspension Cultured Animal Cells

Transfer cells (3-5 x 10^7) to a 15 ml centrifuge tube then centrifuge for 5 minutes at 300 x g. Discard the supernatant retaining approximately 50 µl of residual buffer and cell pellet. Vortex the tube until the cell pellet is completely resuspended in the residual buffer.

2. Lysis

Add 6 ml of Cell Lysis Buffer to the tube then mix by vortex. Incubate at 60°C for at least 10 minutes to ensure the sample lysate is clear/homogenous. During incubation, invert the tube every 3 minutes.

Optional RNA Removal Step.

Following 60°C incubation, add 30 µl of RNase A (10 mg/ml) to the sample then mix by vortex. Incubate at room temperature for 5 minutes.

3. Protein Removal

Add 2 ml of Protein Removal Buffer then vortex immediately for 10 seconds. Centrifuge at 2,000-3,000 x g for 5 minutes to form a tight pellet.

NOTE! If the pellet is loose then incubate on ice for 5 minutes followed by centrifugation at 3,000-6,000 x g for another 5 minutes.

4. DNA Precipitation

Transfer the supernatant to a new 15 ml centrifuge tube then add 6 ml of isopropanol and mix well by gently inverting 20 times. Centrifuge at 2,000-3,000 x g for 5 minutes then carefully discard the supernatant and add 6 ml of 70% ethanol to wash the pellet. Centrifuge at 2,000-3,000 x g for 3 minutes then carefully discard the supernatant and air-dry the pellet for 10 minutes.

5. DNA Rehydration

Add 200 µl of DNA Hydration Buffer then gently vortex for 10 seconds. Incubate at 60°C for 30-60 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.

Troubleshooting

Improper sample preparation.

Adherent Cultured Animal Cells (trypsinize cells prior to harvesting)

Remove the culture medium and wash cells in PBS. Aspirate PBS and add 0.10-0.25% Trypsin in PBS. Once cells detach add the medium then transfer to a 1.5 ml microcentrifuge tube. Proceed with Suspension Cultured Animal cells.

Suspension Cultured Animal Cells

Transfer cells (3-5 x 10^6) to a 1.5 ml microcentrifuge tube then centrifuge for 5 minutes at 300 x g. Discard the supernatant retaining approximately 50 µl of residual buffer and cell pellet. Vortex the tube until the cell pellet is completely resuspended in the residual buffer.

Incomplete protein removal.

A solid protein pellet must be formed following centrifugation in Step 3. If the pellet is loose then incubate on ice for 5 minutes followed by centrifugation at 2,000-3,000 x g for another 3 minutes.

RNA contamination.

Perform the optional RNA removal step. Following 60ºC incubation, add 30 µl of RNase A (10 mg/ml) to the sample then mix by vortex. Incubate at room temperature for 5 minutes.

Slow DNA rehydration.

In step 5, tap the bottom of the tube occasionally to facilitate DNA rehydration. If the DNA pellet is too dry, incubate at 60ºC for 60 minutes or at room temperature overnight.

Eluted DNA does not perform well in downstream applications.

Increase DNA pellet drying time to ensure residual ethanol is completely evaporated.

Related DNA Extraction Products

Plasmid DNA Purification		
Product	Package Size	Catalogue Number
I-Blue Midi Plasmid Kit	50/300 preps	IB47221/222
I-Blue Midi Plasmid Kit	100/300 preps	IB47281/282
I-Blue Midi Plasmid Kit (Endotoxin Free)	100 preps	IB47230
Fast Ion Plasmid Midi Kit	100/300 preps	IB47276/277
Fast Ion Plasmid Midi Kit (Endotoxin Free)	100/300 preps	IB47291/292
Fast Ion Plasmid Maxi Kit	4/10 x 96 preps	IB47251/252
Fast Ion Plasmid Maxi Kit (Endotoxin Free)	100/1000 rxns	IB47431/432
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
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
gSWAB Mini Genomic DNA Kit	100/300 preps	IB47276/277
Genomic DNA Maxi Kit (Plant)	10/25 preps	IB47240/241
gBAC Mini DNA Bacteria Kit	100/300 preps	IB47291/292

96-Well Genomic DNA Extraction Kit	4/10 x 96 preps	IB47251/252
gPURE Cell DNA Isolation Kit	100/1000 rxns	IB47431/432
96-Well Genomic DNA Extraction Kit (Plant)	4/10 x 96 preps	IB47271/272
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
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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