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IBI SCIENTIFIC

INSTRUCTION MANUAL

Genomic DNA Mini Kit (Blood/CulturedCell)

IB47200
IB47201
IB47202

Sample: up to 300 µl of whole fresh blood, up to 1 x 10^7 cultured animal cells, up to 200 µl of buffy coat, up to 2 x 10^8 yeast/fungus

Yield: 4-6 µg from 200 µl of whole blood

Format: spin column

Time: within 25 minutes

Elution volume: 30-200 µl

Storage: dry at room temperature (15-25°C)

Introduction

The Genomic DNA Mini Kit (Blood/Cultured Cell) provides an efficient method for purifying total DNA (including genomic, mitochondrial and viral DNA) from whole fresh blood, cultured animal cells, buffy coat, yeast and other fungus species. RBC Lysis Buffer and chaotropic salt are used to lyse cells and degrade protein, allowing DNA to bind to the glass fiber matrix of the spin column. Contaminants are removed using a Wash Buffer (containing ethanol) and the purified genomic DNA is eluted by a low salt Elution Buffer, TE or water. The entire procedure can be completed within 25 minutes without phenol/chloroform extraction or alcohol precipitation. The purified DNA, with approximately 20-30 kb, is suitable for use in PCR or other enzymatic reactions.

Quality Control

The quality of the Genomic DNA Mini Kit (Blood/Cultured Cell) is tested on a lot-to-lot basis by isolating genomic DNA from 200 µl of whole fresh human blood. The purified DNA (4-6 µg with an A260/A280 ratio of 1.6-1.8) is quantified with a spectrophotometer and analyzed by electrophoresis.

Kit Contents

Component	IB47200	IB47201	IB47202
RBC Lysis Buffer	6 ml	135 ml	135 ml x 3
GT Buffer	1.5 ml	30 ml	75 ml
GB Buffer	2 ml	40 ml	100 ml
W1 Buffer	2 ml	45 ml	130 ml
Wash Buffer* (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
Elution Buffer	1 ml	30 ml	75 ml
GD Columns	4	100	300
2 ml Collection Tubes	8	200	600

* Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use.

Order Information

Genomic DNA Extraction		
Product	**Package Size**	**Catalogue #**
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	IB47201/202
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

Product	Package Size	Catalogue #
gSWAB Mini Genomic DNA Kit	100/300 preps	IB47276/277
gBAC Mini DNA Bacteria Kit	100/300 preps	IB47291/292
gYEAST Genomic DNA Kit	100/300 preps	IB47266/267
96 Well Blood Genomic DNA Extraction Kit	4/10 x 96 preps	IB47251/252
gPURE Cell DNA Isolation Kit	100/1000 rxns	IB47431/432

Caution

GB Buffer contains guanidine hydrochloride. During operation, always wear a lab coat, disposable gloves, and protective goggles.

Genomic DNA Mini Kit (Blood/Cultured Cell) Functional Test Data

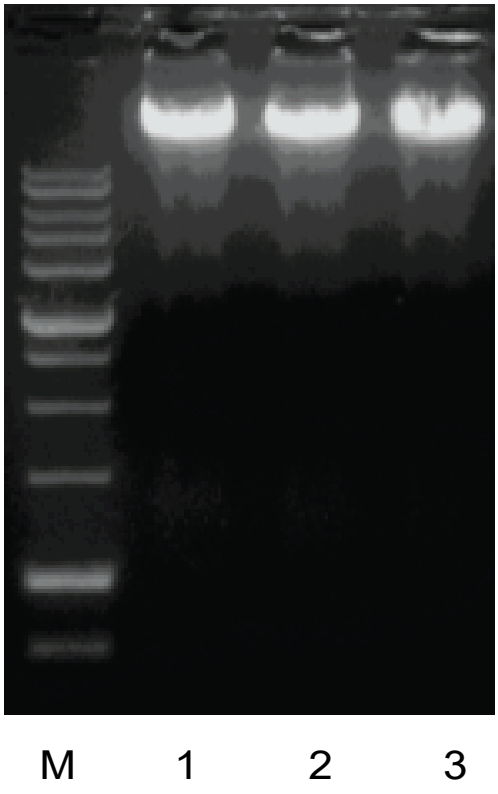
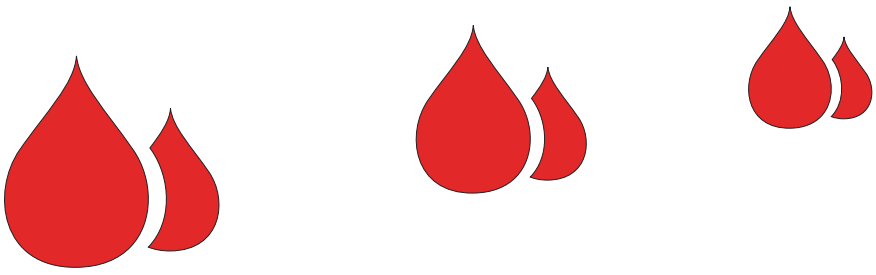


Figure 1. Genomic DNA (30-40 kb) was extracted from 300 µl of whole blood using the Genomic DNA Mini Kit (Blood/Cultured Cell). The purified DNA was eluted in 200 µl of Elution Buffer and 15 µl aliquots of the final sample were analyzed by electrophoresis on a 1% agarose gel.

M = 1 Kb DNA Ladder



Genomic DNA Mini Kit (Blood/Cultured Cell) Fresh Blood Protocol

IMPORTANT BEFORE USE!

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Collect blood in EDTA-NA_2 treated tubes (or other anticoagulant mixtures)
- Additional requirements: microcentrifuge tubes, centrifuge tube, absolute ethanol, (optional) RNase A (10 mg/ml)

Sample Preparation	• Transfer up to 300 µl of blood to a 1.5 ml microcentrifuge tube. NOTE: If the blood sample is more than 300 µl (up to 1 ml), add to a sterile 15 ml centrifuge tube. • Add 3X the sample volume of RBC Lysis Buffer then mix by inversion. Do not vortex. • Incubate the tube for 10 minutes at room temperature. • Centrifuge for 5 minutes at 3,000 x g then remove the supernatant completely. • Add 100 µl of RBC Lysis Buffer to resuspend the leukocyte pellet then proceed with Cell Lysis.
Step 1 Cell Lysis	Add 200 µl of GB Buffer then shake the 1.5 ml microcentrifuge tube vigorously. • Incubate at 60°C for at least 10 minutes to ensure the sample lysate is clear. • During incubation, invert the tube every 3 minutes. At this time, preheat the required Elution Buffer (200 µl per sample) to 60°C (for Step 4 DNA Elution). Optional Step: RNA Degradation (If RNA-free gDNA is required, perform this optional step) • Following 60°C incubation, add 5 µl of RNase A (10 mg/ml) to the clear lysate then mix by shaking vigorously. • Incubate at room temperature for 5 minutes.
Step 2 DNA Binding	• Add 200 µl of absolute ethanol to the lysate then immediately mix by shaking vigorously for 10 seconds. NOTE: If precipitate appears, break it up as much as possible with a pipette. • Place a GD Column in a 2 ml Collection Tube. • Transfer the mixture (including any precipitate) to the GD Column then centrifuge at 14-16,000 x g for 5 minutes. • Discard the 2 ml Collection Tube then place the GD Column in a new 2 ml Collection Tube.
Step 3 Wash	Add 400 µl of W1 Buffer to the GD Column then centrifuge at 14-16,000 x g for 30-60 seconds. • Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. • Add 600 µl of Wash Buffer (make sure ethanol was added) to the GD Column. • Centrifuge at 14-16,000 x g for 30-60 seconds then discard the flow-through. • Place the GD Column back in the 2 ml Collection Tube. • Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.
Step 4 DNA Elution	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl. • Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. • Add 100 µl of pre-heated Elution Buffer, TE or water to the CENTER of the column matrix. • Let stand for at least 3 minutes to ensure the Elution Buffer, TE or water is completely absorbed. • Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.

Genomic DNA Mini Kit (Blood/Cultured Cell) Buffy Coat Protocol

IMPORTANT BEFORE USE!

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: microcentrifuge tubes, centrifuge tube, absolute ethanol, (optional) RNase A (10 mg/ml)

Sample Preparation	Transfer up to 200 µl of buffy coat to a 1.5 ml microcentrifuge tube. • Add 3X the sample volume of RBC Lysis Buffer then mix the tube by inversion. • Incubate the tube for 10 minutes at room temperature. During incubation, invert the tube every 3 minutes. • Centrifuge at 14-16,000 x g for 1 minute then discard the supernatant completely.

Sample Preparation	• Add 500 µl of RBC Lysis Buffer to resuspend the leukocyte pellet. • Centrifuge at 14-16,000 x g for 1 minute then discard the supernatant completely. • Add 200 µl of RBC Lysis Buffer to the tube then resuspend the leukocyte pellet completely. NOTE: Mix the tube by vortex only if the pellet is not resuspended completely and the column becomes barred.
Step 1 Cell Lysis	• Add 250 µl of GB Buffer then shake the tube vigorously. • Incubate at 60ºC for at least 30 minutes to ensure the sample lysate is clear (invert the tube every 3 minutes). At this time, preheat the required Elution Buffer (200 µl per sample) to 60ºC (for Step 4 DNA Elution). Optional Step: RNA Degradation (If RNA-free gDNA is required, perform this optional step) • Following 60ºC incubation, add 5 µl of RNase A (10 mg/ml) to the clear lysate then mix by shaking vigorously. • Incubate at room temperature for 5 minutes.
Step 2 DNA Binding	• Add 250 µl of absolute ethanol to the lysate then immediately mix by shaking vigorously for 10 seconds. NOTE: If precipitate appears, break it up as much as possible with a pipette. • Place a GD Column in a 2 ml Collection Tube. • Transfer the mixture (including any precipitate) to the GD Column and centrifuge 14-16,000 x g for 5 minutes. • Discard the 2 ml Collection Tube then place the GD Column in a new 2 ml Collection Tube.
Step 3 Wash	• Add 400 µl of W1 Buffer to the GD Column then centrifuge at 14-16,000 x g for 30-60 seconds. • Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. • Add 600 µl of Wash Buffer (make sure ethanol was added) to the GD Column. • Centrifuge at 14-16,000 x g for 30-60 seconds then discard the flow-through. • Place the GD Column back in the 2 ml Collection Tube. • Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.
Step 4 DNA Elution	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl. • Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. • Add 100 µl of pre-heated Elution Buffer, TE or water to the CENTER of the column matrix. • Let stand for at least 10 minutes to ensure the Elution Buffer, TE or water is completely absorbed. • Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.

Genomic DNA Mini Kit (Blood/Cultured Cell) Cultured Cell Protocol

IMPORTANT BEFORE USE!

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: microcentrifuge tubes, centrifuge tube, absolute ethanol, phosphate-buffered saline (PBS), 0.10-0.25% Trypsin, (optional) RNase A (10 mg/ml)

Sample Preparation	Adherent Cultured Animal Cells (trypsinize cells prior to harvesting) 1. Remove the culture medium and wash cells in PBS. Aspirate PBS and add 0.10-0.25% Trypsin in PBS. 2. Once cells detach add medium then transfer to a 15 ml centrifuge tube. 3. Proceed with Suspension Cultured Animal cells. Suspension Cultured Animal Cells • Transfer cells (up to 1 x 10^7) to a 1.5 ml microcentrifuge tube then centrifuge for 5 minutes at 300 x g. • Discard the supernatant then resuspend cells in 150 µl of RBC Lysis Buffer by pipette. Proceed with Step 1. Up to 50 µl of non-nucleated mammalian blood or up to 10 µl of nucleated erythrocytes (e.g. bird or fish) • Add 150 µl of GT Buffer and blood sample to a 1.5 ml microcentrifuge tube then shake vigorously.
Step 1 Cell Lysis	• Add 200 µl of GB Buffer to the 1.5 ml microcentrifuge tube then shake vigorously. • Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear.

Step 1 Cell Lysis	• During incubation, invert the tube every 3 minutes. At this time, preheat the required Elution Buffer (200 µl per sample) to 60°C (for Step 4 DNA Elution). Optional Step: RNA Degradation (If RNA-free gDNA is required, perform this optional step) • Following 60°C incubation, add 5 µl of RNase A (10 mg/ml) to the clear lysate then mix by shaking vigorously. • Incubate at room temperature for 5 minutes.
Step 2 DNA Binding	• Add 200 µl of absolute ethanol to the lysate then immediately mix by shaking vigorously for 10 seconds. NOTE: If precipitate appears, break it up as much as possible with a pipette. • Place a GD Column in a 2 ml Collection Tube. • Transfer the mixture (including any precipitate) to the GD Column then centrifuge at 14-16,000 x g for 2 minutes. • Discard the 2 ml Collection Tube then place the GD Column in a new 2 ml Collection Tube.
Step 3 Wash	• Add 400 µl of W1 Buffer to the GD Column then centrifuge at 14-16,000 x g for 30-60 seconds. • Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. • Add 600 µl of Wash Buffer (make sure ethanol was added) to the GD Column. • Centrifuge at 14-16,000 x g for 30-60 seconds then discard the flow-through. • Place the GD Column back in the 2 ml Collection Tube. • Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.
Step 4 DNA Elution	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl. • Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. • Add 100 µl of pre-heated Elution Buffer, TE or water to the CENTER of the column matrix. • Let stand for at least 3 minutes to ensure the Elution Buffer or TE is completely absorbed. • Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.

Genomic DNA Mini Kit (Blood/Cultured Cell) Yeast/Fungus Protocol

IMPORTANT BEFORE USE!

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: microcentrifuge tubes, absolute ethanol, lyticase or zymolase, 50 mM EDTA pH8.0, (optional) RNase A (10 mg/ml)

Cell Harvesting Pre-Lysis	• Transfer yeast/fungus cells (up to 2 x 10^8) to a 1.5 ml microcentrifuge tube. • Harvest cells by centrifugation for 10 minutes at 5,000 x g. • Discard the supernatant and resuspend the pellet in 600 µl of 50 mM EDTA pH8.0. • Add 200 U of lyticase or zymolase then incubate at 30°C for 30 minutes. • Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast. • Remove the supernatant then add 200 µl of GT Buffer. • Resuspend the cell pellet by shaking vigorously or pipetting then incubate at room temperature for 5 minutes.
Step 1 Cell Lysis	• Add 200 µl of GB Buffer to the sample and mix the tube by shaking vigorously for 5 seconds. • Incubate at 60°C for 10 minutes to ensure the sample lysate is clear. NOTE: During incubation, invert the tube every 3 minutes. At this time, preheat the required Elution Buffer (200 µl per sample) to 60°C (for Step 4 DNA Elution). Optional Step: RNA Degradation (If RNA-free gDNA is required, perform this optional step) • Following 60°C incubation, add 5 µl of RNase A (50 mg/ml) to the clear lysate then mix by shaking vigorously. • Incubate at room temperature for 10 minutes.
Step 2 DNA Binding	• Add 200 µl of absolute ethanol to the sample lysate then immediately mix by shaking vigorously. NOTE: If precipitate appears, break it up as much as possible with a pipette.

Step 2 DNA Binding	• Place a GD Column in a 2 ml Collection Tube. • Transfer the mixture (including any precipitate) to the GD Column then centrifuge at 14-16,000 x g for 2 minutes. • Discard the 2 ml Collection Tube and flow-through then place the GD Column in a new 2 ml Collection Tube.
Step 3 Wash	• Add 400 µl of W1 Buffer to the GD Column then centrifuge at 14-16,000 x g for 30 seconds • Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. • Add 600 µl of Wash Buffer (make sure ethanol was added) to the GD Column. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through then place the GD Column back in the 2 ml Collection Tube. • Centrifuge for 3 minutes at 14-16,000 x g to dry the column matrix.
Step 4 DNA Elution	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl. • Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. • Add 100 µl of pre-heated Elution Buffer, TE or water to the CENTER of the column matrix. • Let stand for at least 3 minutes to ensure the Elution Buffer, TE or water is completely absorbed. • Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.

Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	• Reduce sample volume or separate into multiple tubes.
Low Yield	• Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Close the bottle tightly after each use to avoid ethanol evaporation. • Reduce the sample material. • Following ethanol addition, break up any precipitate as much as possible prior to loading GD Column. • Add Elution Buffer, TE or water is added to the CENTER of the GD Column and is absorbed completely. • Elute twice to increase yield.
Eluted DNA does not perform well in downstream applications	• Following the Wash Step, dry the GD Column by centrifuge at 14-16,000 x g for 5 minutes or incubate at 60ºC for 5 minutes. • Use fresh blood as long term storage may result in fragmentation of genomic DNA. • Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. • If using water for elution, ensure the water pH is between 7.5 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. DNA eluted in water should be stored at -20ºC to avoid degradation.

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