For Research Use Only

IBI SCIENTIFIC

INSTRUCTION MANUAL

96 Well Blood Genomic DNA Extraction Kit

IB47250 (2 x 96 well plates/kit)

IB47251 (4 x 96 well plates/kit)

IB47252 (10 x 96 well plates/kit)

Advantages

Sample: up to 200 µl of whole blood, plasma, serum and up to 5 x 10^6 lymphocytes or cultured cells per well
gDNA Yield: up to 6 µg of genomic DNA from 200 µl of whole blood
Format: 96 Well gDNA Binding Plate
Time: 45 minutes
Elution Volume: 200 µl
Kit Storage: dry at room temperature (15-25°C)

Introduction

The 96 Well Blood Genomic DNA Extraction Kit was designed for high-throughput purification of genomic, mitochondrial and virus DNA from whole blood (fresh blood and frozen blood), plasma, serum, body fluids, lymphocytes and cultured cells. This DNA extraction kit uses protease and chaotropic salt to lyse cells and degrade protein, allowing DNA to bind to the glass fiber matrix of the 96 Well gDNA Binding Plate. Contaminants are removed using a Wash Buffer and the purified genomic DNA is eluted by a low salt Elution Buffer, TE or water. The procedure can be completed within 45 minutes without phenol/chloroform extraction or alcohol precipitation. The purified DNA (approximately 20-30 kb) is suitable for use in PCR or other enzymatic reactions.

Quality Control

The quality of the 96 Well Blood Genomic DNA Extraction Kit is tested on a lot-to-lot basis by purifying genomic DNA from 200 µl of whole blood samples. The purified DNA is quantified with a spectrophotometer and analyzed by electrophoresis.

Kit Contents

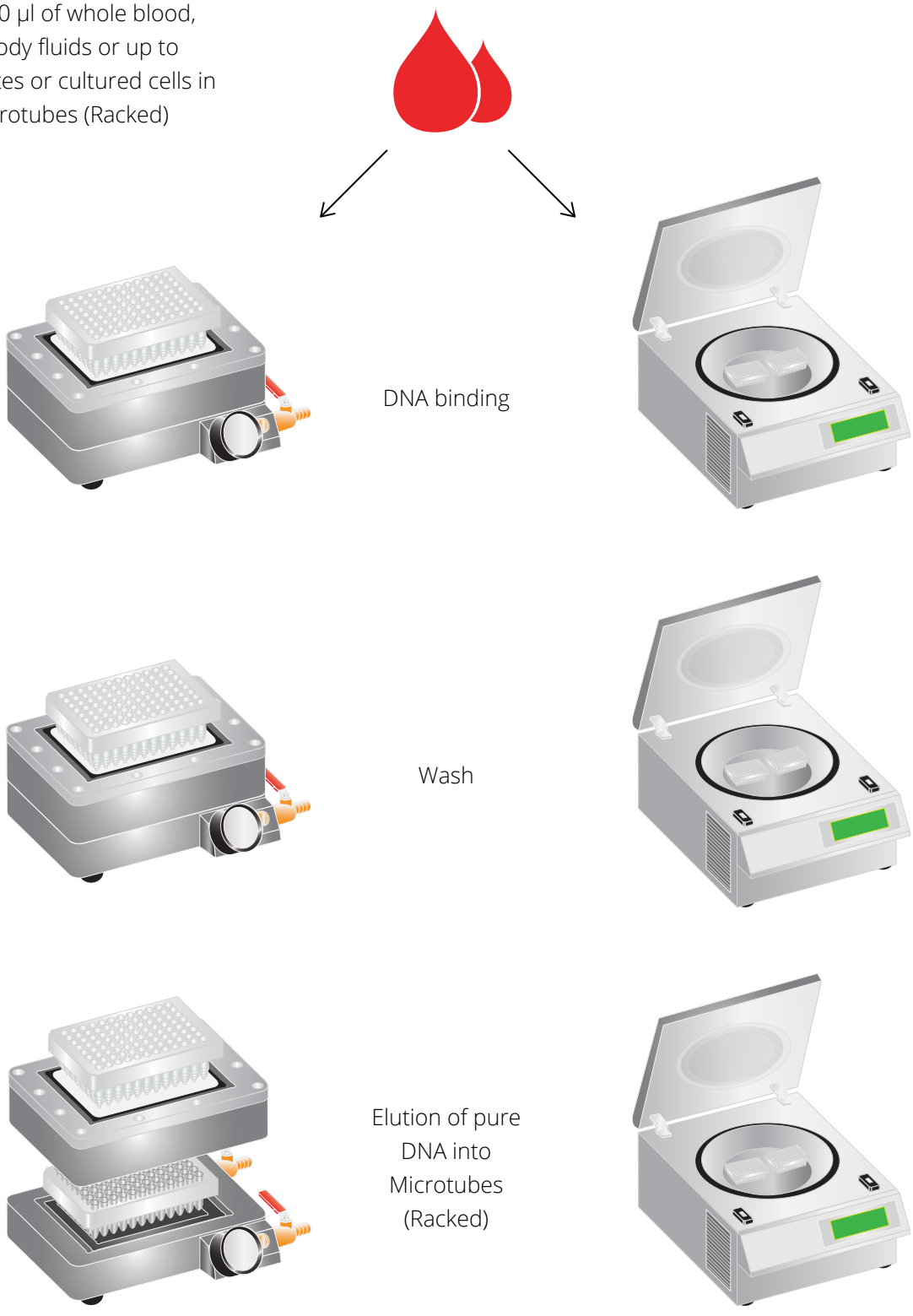
Component	IB47250	IB47251	IB47252
GB Buffer	40 ml	100 ml	155 ml x 1
			60 ml x 1
Protease [1]	4 ml	8 ml	20 ml
W1 Buffer	80 ml	200 ml	200 ml x 2
Wash Buffer [2] (Add Ethanol)	25 ml (100 ml)	50 ml (200 ml)	25 ml x 1 (100 ml)
			50 ml x 2 (200 ml x 2)
Elution Buffer	100 ml	100 ml x 2	100 ml x 4
96 Well gDNA 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-strip)	72	72 x 2	72 x 5
96 Deep Well Plates [3]	2	2	2

[1] Protease is shipped at room temperature and should be stored at 2-8°C for up to 6 months.
[2] 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. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.
[3] 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 ddH2O. The plate can be autoclaved after being washed.

IMPORTANT BEFORE USE! **During the procedure, always wear a lab coat, disposable gloves, and protective goggles.**

Quick Protocol Diagram

Transfer up to 200 µl of whole blood, plasma, serum, body fluids or up to 5 x 10^6 lymphocytes or cultured cells in 200 µl PBS to Microtubes (Racked)



96 Well Blood gDNA Kit Protocol

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

IMPORTANT BEFORE USE!

1. Protease is shipped at room temperature and should be stored at 2-8°C for up to 6 months.
2. 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. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.
3. 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 ddH2O. The plate can be autoclaved after being washed.

Additional Requirements:
Centrifuge with microplate buckets, 70°C oven or incubator

Centrifuge Protocol Procedure

1. Sample Preparation and Lysis

Transfer 20 µl of Protease to the bottom of each microtube in Microtubes (Racked). Transfer up to 200 µl of whole blood, plasma, serum, body fluids or up to 5 x 10^6 lymphocytes or cultured cells in 200 µl PBS to the Microtubes (Racked), being careful not to touch the rims of the Microtubes (Racked) with the pipette tips.

NOTE: If the sample volume is less than 200 µl, use PBS to adjust the volume to 200 µl.

Add 200 µl of GB Buffer to each sample, being careful not to touch the rims of the microtubes with the pipette tips. If RNA-free genomic DNA is required, add 5 µl of RNase A (50 mg/ml) to each sample. Seal the microtubes with the Microtube Caps (8-strip). Cover the rack with the plastic lid then mix the sample thoroughly by shaking vigorously for 15 seconds. Incubate the Microtubes (Racked) at 70°C in an oven or incubator for at least 10 minutes.

NOTE: At this time, pre-heat the required Elution Buffer (400 µl per sample) to 60°C (for Step 4 DNA Elution).

2. DNA Binding

Briefly centrifuge the Microtubes (Racked) at 2,000 x g to collect any lysate from the caps. Allow the centrifuge to reach 2,000 x g prior to stopping. Remove the caps and add 200 µl of absolute ethanol to each sample. Seal the Microtubes (Racked) with new caps. Cover the rack with the plastic lid then shake vigorously for 15 seconds. Briefly centrifuge the Microtubes (Racked) at 2,000 x g to collect any lysate from the caps. Allow the centrifuge to reach 2,000 x g prior to stopping. Place a 96 Well gDNA Binding Plate on a 96 Deep Well Plate. Remove the caps from the Microtubes (Racked) then transfer the lysate to each well of the 96 Well gDNA Binding Plate, being careful not to get any lysate on the the rims of the wells. Centrifuge the 96 Well gDNA Binding Plate and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through. Place the 96 Well gDNA Binding Plate back on the 96 Deep Well Plate.

3. Wash

Add 400 µl of W1 Buffer to each well of the 96 Well gDNA Binding Plate then centrifuge the 96 Well gDNA Binding Plate and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through. Place the 96 Well gDNA Binding Plate back on the 96 Deep Well Plate. Add 600 µl of Wash Buffer (make sure ethanol was added) to each well of the 96 Well gDNA Binding Plate. Centrifuge the 96 Well gDNA Binding Plate and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through. Place the 96 Well gDNA Binding Plate back on the 96 Deep Well Plate. Centrifuge the 96 Well gDNA 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 gDNA 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 gDNA Binding Plate on Microtubes (Racked). Add 200 µl of pre-heated Elution Buffer[1], TE[2] or water[3] to the center of each well of the 96 Well gDNA Binding Plate. Let stand for at least 2 minutes to ensure the Elution Buffer, TE or water is absorbed by the membrane. Centrifuge the 96 Well gDNA Binding Plate and Microtubes (Racked) together at 3,000 x g for 5 minutes to elute the purified DNA. Seal the Microtubes (Racked) with new caps then store the purified DNA at -20°C.

NOTE: For maximum DNA yield, repeat the elution step by adding 200 µl of pre-heated Elution Buffer, TE or water to each well of the 96 Well gDNA Binding Plate then centrifuge again.

[1] Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C) is added into the CENTER of the well matrix and is completely absorbed.

[2] 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. Ensure that TE is added into the CENTER of the well matrix and is completely absorbed.

[3] If using water for elution, ensure the water pH is ≥8.0. ddH2O should be fresh as ambient CO2 can quickly cause acidification. Ensure that water is added into the CENTER of the well matrix and is completely absorbed. DNA eluted in water should be stored at -20°C to avoid degradation.

Vacuum Protocol Procedure

1. Vacuum Manifold Preparation

Place the waste tray on the manifold base then place the binding top plate on the manifold base. Place the 96 Well gDNA Binding Plate in the binding top plate aperture. Attach the vacuum manifold to a vacuum source.

2. Sample Preparation and Lysis

Transfer 20 µl of Protease to the bottom of each microtube in a Microtubes (Racked). Transfer up to 200 µl of whole blood, plasma, serum, body fluids or up to 5 x 10^6 lymphocytes or cultured cells in 200 µl PBS to the Microtubes (Racked), being careful not to touch the rims of the Microtubes (Racked) with the pipette tips.

NOTE: If the sample volume is less than 200 µl, use PBS to adjust the volume to 200 µl. Add 200 µl of GB Buffer to each sample, being careful not to touch the rims of the microtubes with the pipette tips. If RNA-free genomic DNA is required, add 5 µl of RNase A (50 mg/ml) to each sample. Mix the samples by pipetting up and down 5 times then seal the microtubes with the Microtube Caps (8-strip). Incubate the Microtubes (Racked) at 70°C in an oven or incubator for at least 10 minutes.

NOTE: At this time, pre-heat the required Elution Buffer (400 µl per sample) to 60°C (for Step 5 DNA Elution).

3. DNA Binding

Remove the caps and add 200 µl of absolute ethanol to each sample then mix well by pipetting up and down 5 times. Transfer the lysate to each well of the 96 Well gDNA Binding Plate, being careful not to get any lysate on the the rims of the wells.

NOTE: Seal unused wells of the 96 Well gDNA Binding Plate with adhesive film. Apply vacuum at 15 inches Hg until the samples pass through the 96 Well gDNA Binding Plate then switch off the vacuum.

4. Wash

Add 400 µl of W1 Buffer to each well of the 96 Well gDNA Binding Plate. Apply vacuum at 15 inches Hg until W1 Buffer passes through the 96 Well gDNA Binding Plate (approximately 10 seconds) then switch off the vacuum. Add 600 µl of Wash Buffer (make sure ethanol was added) to each well of the 96 Well gDNA Binding Plate. Apply vacuum at 15 inches Hg until Wash Buffer passes through the 96 Well gDNA Binding Plate. Continue to apply vacuum for an additional 10 minutes to dry the membrane then switch off the vacuum.

5. Elution

Remove the 96 Well gDNA Binding Plate from the manifold and blot the nozzles on clean, absorbent paper towel to remove residual ethanol. Remove the waste tray from the manifold base then place Microtubes (Racked) on the manifold base. Place the binding top plate on the manifold base then place the 96 Well gDNA Binding Plate in the binding top plate aperture.

Add 200 µl of pre-heated Elution Buffer[1], TE[2] or water[3] to the CENTER of each well of the 96 Well gDNA Binding Plate. Let stand for at least 2 minutes to ensure the Elution Buffer, TE or water is absorbed by the membrane. Apply vacuum at 15 inches Hg for 5 minutes to elute the purified DNA. Seal the Microtubes (Racked) with new caps, then store the purified DNA at -20°C.
NOTE: For maximum DNA yield, repeat the elution step by adding 200 µl of pre-heated Elution Buffer, TE or water to each well of the 96 Well gDNA Binding Plate then apply vacuum at 15 inches Hg for 5 minutes again.

[1] Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C) is added into the CENTER of the well matrix and is completely absorbed.
[2] 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. Ensure that TE is added into the CENTER of the well matrix and is completely absorbed.
[3] If using water for elution, ensure the water pH is ≥8.0. ddH2O should be fresh as ambient CO2 can quickly cause acidification. Ensure that water is added into the CENTER of the well matrix and is completely absorbed. DNA eluted in water should be stored at -20°C to avoid degradation.

96 Well Blood Genomic DNA Kit Functional Test Data

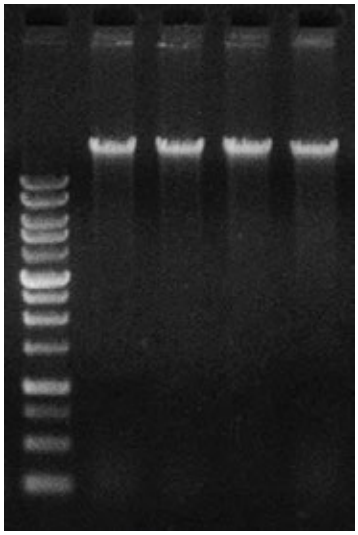


Figure 1.
Genomic DNA was extracted from 200 µl whole human blood samples using the 96 Well Blood Genomic DNA Extraction Kit. The purified genomic DNA was eluted in 200 µl of Elution Buffer and 15 µl aliquots of the final sample (chosen from 4 random wells) were analyzed by electrophoresis on a 1% agarose gel.
M = 1 Kb DNA Ladder

Sample	ng/µl	260/280	Yield
1. 200 µl blood	27.6	1.81	4.7 µg
2. 200 µl blood	28.2	1.75	4.8 µg
3. 200 µl blood	32.9	1.74	5.6 µg
4. 200 µl blood	25.9	1.81	4.4 µg

Troubleshooting

Low Yield

DNA degradation due to improper storage of blood samples.
Yield and quality of DNA will be higher when fresh blood is used. Whole blood samples in anticoagulant treated tubes can be stored for several weeks at 4°C. However, frozen blood can also be used. Increased storage length decreases DNA yield.

Incomplete buffer preparation.
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. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Incomplete sample preparation.
After adding Protease, samples and GB Buffer into microtubes, mix samples thoroughly by shaking vigorously or pipetting. DO NOT add protease directly to GB Buffer. Store Protease at 2-8°C for up to 6 months.

Clogged column.
Use the recommended amount of starting material. Overloading the columns will cause clogging and low DNA yield.

Incorrect DNA elution step.
Ensure that Elution Buffer, TE or water is added into the CENTER of the matrix and is completely absorbed. Use pre-heated Elution Buffer, TE, or water (60°C). If using water for elution, ensure the water pH is between 7.5 and 8.5. ddH2O should be fresh as ambient CO2 can quickly cause acidification. Elute twice to increase the DNA recovery.

Eluted DNA Does Not Perform Well In Downstream Applications

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

Residual RNA Contamination.
Perform the optional RNA removal step during sample preparation and lysis.

Related DNA/RNA Extraction Products

Total RNA Extraction		
Product	**Package Size**	**Catalogue Number**
Total RNA Mini Kit (Blood/Cell)	50/100/300 preps	IB47321/322/323
Total RNA Mini Kit (Tissue)	50/100/300 preps	IB47301/302/303
Total RNA Mini Kit (Plant)	50/100/300 preps	IB47341/342/343
rBAC Mini RNA Bacteria Kit	100/300 preps	IB47421/422
rYeast Total RNA Mini Kit	100/300 preps	IB47411/412
miRNA Isolation Kit	100 preps	IB47371
IBI Isolate	100/200 rxns	IB47601/302
Plasmid DNA Extraction		
Product	**Package Size**	**Catalogue Number**
I-Blue Mini Plasmid Kit	100/300 preps	IB47171/172
I-Blue Mini Plasmid Endo Free Kit	100 preps	IB47176
I-Blue Midi Plasmid Kit	25 preps	IB47181
I-Blue Midi Plasmid Kit	25 preps	IB47191
Midi Fast Ion Plasmid Kit	25 preps	IB47111
Midi Fast Ion Plasmid Kit (Endotoxin Free)	25 preps	IB47113
Maxi Fast Ion Plasmid Kit	10/25 preps	IB47121/122
Maxi Fast Ion Plasmid Kit (Endotoxin Free)	10/25 preps	IB47124/125
96 Well Plasmid Kit	4/10 x 96 preps	IB47151/152
Post Reaction DNA Extraction		
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 PCR Cleanup Kit	4/10 x 96 preps	IB47040/050
Genomic DNA Extraction		
Product	**Package Size**	**Catalogue Number**
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
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
DNA/RNA/Protein Extraction		
Product	**Package Size**	**Catalogue Number**
DNA/RNA/Protein Extraction Kit	50/100 preps	IB47701/702

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