96-Well Genomic DNA Kit

For research use only

Sample size : up to 200 µl of blood, up to 20 mg of tissue, cultured animal cells (up to 1 x 10^7), bacterial cells (up to 1 x 10^9) and fungus cells (up to 5 x 10^7) Hair, Blood Spot, Semen

Yield : up to 30 µg/well

Format : 96-well plates

Operation : centrifuge

Applications : PCR, AFLP, RFLP, Southern Blotting, Real-time PCR

www.ibisci.com

Revised: 5/20/10

Introduction

The 96-Well Genomic DNA Kit was designed for high-throughput purification of total DNA (including genomic, mitochondrial and viral DNA) from a variety of samples. Proteinase K and chaotropic salt are used to lyse cells and degrade protein, allowing DNA in the chaotropic salt to be easily bound by the glass fiber matrix (1) of each well of the plate. Once any contaminants have been removed, the purified DNA is eluted by a low salt Elution Buffer or TE. The entire procedure can be completed in 1 hour without phenol/chloroform extraction or alcohol precipitation. The kits can be used for manual filtration or with robotic handling systems and the purified DNA with approximately 20-30 Kb is suitable for use in PCR or other enzymatic reactions.

Quality Control

The quality of the 96-Well Genomic DNA Kit is tested on a lot-to-lot basis by isolating genomic DNA from 200 µl of fresh whole human blood. The purified DNA (4-6 µg with an A260/A280 ratio of 1.6 - 1.8) is quantified with a spectrophotometer and checked by electrophoresis.

Kit Contents

Name	IB47250	IB47251	IB47252
GT Buffer	60 ml	120 ml	240 ml
GB Buffer	60 ml	120 ml	240 ml
W1 Buffer	130 ml	130 ml	130 ml x 3
Wash Buffer* (Add Ethanol)	25 ml (100 ml)	50 ml (200 ml)	50 ml (200 ml x 3)
Elution Buffer	30 ml	60 ml	120 ml
Proteinase K** (Add ddH_20)	45 mg (4.5 ml)	45 mg x 2 (4.5 ml x 2)	45 mg x 5 (4.5 ml x 5)
DNA Binding Plate	2 pcs	4 pcs	10 pcs
0.35 ml Collection Plate	2 pcs	4 pcs	10 pcs
Adhesive film	4 pcs	8 pcs	20 pcs

Order Information

Product Name	Package size	Cat. No.
Genomic DNA Mini Kit (Tissue)	50/300 preps	IB47221/222
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	IB47201/202
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10 preps	IB47210
IBI Plant Isolate	100/500 ml	IB47611/612
Genomic DNA Mini Kit (Plant)	100 preps	IB47231
Genomic DNA Maxi Kit (Plant)	10/25 preps	IB47240/241
96-Well Genomic Plant DNA Kit	4/10 x 96 Wells	IB47261/262
96-Well Genomic DNA Kit	4/10 x 96 Wells	IB47251/252
96-Well Genomic DNA Kit (Plant)	4/10 x 96 Wells	IB47271/272

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

**Add ddH_20 (see the bottle label for volume) to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4ºC

Caution

GB Buffer and W1 Buffer contain guanidine hydrochloride, which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615

96-Well Genomic DNA Kit Blood Protocol

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Add ddH_20 (see the bottle label for volume) to prepare the Proteinase K (vortex to dissolve and spin down) and store at 4ºC
- Additional requirements: centrifugation system for 96-well plates, absolute ethanol, 2 ml collection plate, ddH_20, RNase A (10 mg/ml)

Step1 Cell Lysis	● Add 200 µl of fresh blood and **20 µl of Proteinase K** to each well of a 2 ml collection plate. ● Incubate at 60°C for 10 minutes. ● Add **200 µl of GB Buffer** to each well and mix by shaking. ● Incubate at 60°C for 20 minutes or until the sample lysate is clear. At this time, preheat the required **Elution Buffer (100 µl per sample)** to 60°C for DNA Elution step. ● If RNA-free genomic DNA is required, add 5 µl of RNase A to each well and incubate at room temperature for 4 minutes.
Step 2 DNA Binding	● Add 200 µl of absolute ethanol to each sample lysate in the 2 ml collection plate from the previous step. ● Mix immediately by pipetting 5-10 times. ● Place a **DNA Binding Plate** on a new 2 ml collection plate. ● Transfer the lysate mixture to the **DNA Binding Plate**. ● Centrifuge for 5 minutes at 3,000 x g.
Step 3 Wash	● Add **300 µl of W1 Buffer** to each well of the **DNA Binding Plate**. ● Centrifuge for 5 minutes at 3,000 x g. ● Add **600 µl of Wash Buffer** (ethanol added) to each well of the **DNA Binding Plate** to wash again. ● Centrifuge for 5 minutes at 3,000 x g. ● Discard the flow-through and place the **DNA Binding Plate** back on the 2 ml collection plate. ● Centrifuge for 10 minutes at 3,000 x g to remove any ethanol residue.
Step 4 DNA Elution	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (50-100 µl) to increase DNA concentration. ● Transfer the **DNA Binding Plate** to a clean **0.35 ml Collection Plate**. ● Add **100 µl of preheated Elution Buffer** or TE to the center of each well of the **DNA Binding Plate**. ● Let stand for 3 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. ● Centrifuge for 5 minutes at 1,000 x g to elute the purified DNA. ❖ For increased efficiency, repeat the elution steps.

96-Well Genomic DNA Kit Tissue Protocol

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Add ddH_20 (see the bottle label for volume) to prepare the Proteinase K (vortex to dissolve and spin down), store at 4ºC
- Additional requirements: centrifugation system for 96-well plates, 2 ml collection plate, RNase A (10 mg/ml), absolute ethanol

Step1 Cell Lysis	● Add **200 µl of GT Buffer** and **20 µl of Proteinase K** to each well of a 2 ml collection plate**.** ● Cut up to 20 mg of animal tissue (or 0.5 cm of mouse tail) and transfer it to each well of the 2 ml collection plate. ● Incubate the plate with shaking at 60°C for 1-2 hours to lyse the sample. ● Add **200 µl of GB Buffer** to each well and mix by shaking. ● Incubate at 70°C for 20 minutes or until the sample lysate is clear. At this time, preheat the required **Elution Buffer (50 µl per sample)** to 70°C for DNA Elution step. ● If there is insoluble material present following incubation, centrifuge the plate for 5 minutes at full speed and transfer the supernatant to a new 2 ml collection plate. ● If RNA-free genomic DNA is required, add 5 µl of RNase A to each well and incubate at room temperature for 4 minutes.
Step 2 DNA Binding	● Add 200 µl of absolute ethanol to each sample lysate in the 2 ml collection plate from the previous step and mix immediately by pipetting 5-10 times. ● Place a **DNA Binding Plate** on a new 2 ml collection plate. ● Transfer the lysate mixture to the **DNA Binding Plate** and centrifuge for 5 minutes at 3,000 x g.
Step 3 Wash	● Add **300 µl of W1 Buffer** to each well of the **DNA Binding Plate**. ● Centrifuge for 5 minutes at 3,000 x g. ● Add **600 µl of Wash Buffer** (ethanol added) to each well of the **DNA Binding Plate** to wash again. ● Centrifuge for 5 minutes at 3,000 x g. ● Discard the flow-through and place the **DNA Binding Plate** back on the 2 ml collection plate. ● Centrifuge for 10 minutes at 3,000 x g to remove any ethanol residue.
Step 4 DNA Elution	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (50-100 µl) to increase DNA concentration. ● Transfer the **DNA Binding Plate** to a clean **0.35 ml Collection Plate**. ● Add **100 µl of preheated Elution Buffer** or TE to the center of each well of the **DNA Binding Plate**. ● Let stand for 3 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. ● Centrifuge for 5 minutes at 1,000 x g to elute the purified DNA. ❖ For increased efficiency, repeat the elution steps.

96-Well Genomic DNA Kit Cultured Cell Protocol

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: centrifugation system for 96-well plates, Phosphate-Buffered Saline (PBS), 2 ml collection plates, absolute ethanol, RNase A (10 mg/ml)

Step 1 Sample Preparation	**Cultured animal cells** If using adherent cells, trypsinize the cells before harvesting. ● Transfer cells (up to 1 x 10^7) to each well of a 2 ml collection plate and harvest with centrifugation for 50 seconds at 5,000 x g. ● Discard the supernatant and resuspend the cells with 150 µl of PBS.
Step 2 Sample Lysis	● Add **200 µl of GB Buffer** to each well of the 2 ml collection plate and mix by shaking. ● Incubate the 2 ml collection plate at 60°C for 20 minutes or until the sample lysate is clear (during incubation shake the 2 ml collection plate every 2-3 minutes). At this time, preheat the required **Elution Buffer (100 µl per sample)** to 60°C for DNA Elution step. ● If RNA-free genomic DNA is required, add 5 µl of RNase A to each well and incubate at room temperature for 4 minutes.
Step 3 DNA Elution	● Add 200 µl of absolute ethanol to each sample lysate in the 2 ml collection plate from the previous step and mix immediately by pipetting 5-10 times. ● Place a **DNA Binding Plate** on a new 2 ml collection plate. ● Transfer the lysate mixture to the **DNA Binding Plate**. ● Centrifuge for 5 minutes at 3,000 x g.
Step 4 Wash	● Add **300 µl of W1 Buffer** to each well of the **DNA Binding Plate**. ● Centrifuge for 5 minutes at 3,000 x g. ● Add **600 µl of Wash Buffer (ethanol added)** into each well of the **DNA Binding Plate** to wash again. ● Centrifuge for 5 minutes at 3,000 x g. ● Discard the flow-through and place the **DNA Binding Plate** back on the 2 ml collection plate. ● Centrifuge for 10 minutes at 3,000 x g to remove the ethanol residue.
Step 5 DNA Elution	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (50-100 µl) to increase DNA concentration. ● Transfer the **DNA Binding Plate** to a **0.35 ml Collection Plate**. ● Add **100 µl of preheated Elution Buffer** or TE into the center of each well of **DNA BindingPlate**. ● Let stand for 3 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. ● Centrifuge for 5 minutes at 1,000 x g to elute the purified DNA. ❖ For increased efficiency, repeat the elution steps.

96-Well Genomic DNA Kit Bacteria Protocol

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: centrifugation system for 96-well plates, 2 ml collection plate, absolute ethanol, RNase A (10 mg/ml), for gram-positive bacteria: lysozyme buffer (20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% Triton X-100; pH 8.0), prepare fresh lysozyme buffer immediately prior to use

Step 1 Cell Harvesting/ Pre-lysis	**Gram-negative bacteria** ● Transfer cultured bacterial cells (up to 1 x 10^9) to each well of a 2 ml collection plate. ● Centrifuge for 3 minutes at full speed and discard the supernatant. ● Add **200 µl of GT Buffer** to each well and resuspend the cell pellet by pipetting. ● Incubate at room temperature for 5 minutes. ● Proceed with the Lysis Step of the Cultured Cell protocol. **Gram-positive bacteria** ● Transfer cultured bacterial cells (up to 1 x 10^9) to each well of a 2 ml collection plate. ● Centrifuge for 3 minutes at full speed and discard the supernatant. ● Add 200 µl of lysozyme buffer to each well and resuspend the cell pellet by pipetting. ● Incubate at room temperature for 10 minutes. During incubation, shake the 2 ml collection plate every 2-3 minutes. ● Proceed with the Lysis Step of the Cultured Cell Protocol.

96-Well Genomic DNA Kit Fungus Protocol

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: centrifugation system for 96-well plates 2 ml collection plate, RNase A (10 mg/ml), lyticase or zymolase, absolute ethanol, sorbitol buffer (1.2 M sorbitol; 10 mM $CaCl_2$; 0.1 M Tris-HCl pH 7.5; 35 mM mercaptoethanol)

Step 1 Cell Harvesting/ Prelysis	• Harvest fungus cells (up to 5 x 10^7) in each well of a 2 ml collection plate by centrifugation for 10 minutes at 5,000 x g. • Discard the supernatant and resuspend the pellet in 600 µl of sorbitol buffer. • Add 200 U lyticase or zymolase. 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 and add **200 µl of GT Buffer** to each well of a 2 ml collection plate and resuspend the cell pellet by pipetting. • Incubate at room temperature for 5 minutes. • Proceed with the Lysis Step of the Cultured Cell Protocol.

96-Well Genomic DNA Kit Hair Protocol

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: centrifugation system for 96-well plates, 2 ml collection plates, absolute ethanol, RNase A (10 mg/ml)

Step 1 Sample Lysis	• Add 10 hairs, including follicle cells to each well of a 2 ml collection plate. • Add 2**00 µl of GT Buffer and 20 µl of Proteinase K** to each well (making sure the hair is completely submerged) and mix by shaking. • Incubate at 60ºC for 30 minutes to lyse the sample. During incubation, shake the collection plate every 5 minutes. • Add 2**00 µl of GB Buffer** to each well and mix vigorously. • Incubate at 70ºC for 20 minutes. During incubation, shake the collection plate every 5 minutes. At this time, preheat the required **Elution Buffer (100 µl per sample)** to 70ºC for DNA Elution step. • Following incubation, centrifuge for 5 minutes at 3,000 x g and transfer the supernatant to a new 2 ml collection plate. • If RNA-free genomic DNA is required, add 5 µl of RNase A to each well and incubate at room temperature for 4 minutes.
Step 2 DNA Binding	• Add 200 µl of absolute ethanol to each sample lysate in the 2 ml collection plate from the previous step. • Mix immediately by pipetting 5-10 times. • Place a **DNA Binding Plate** on a new 2 ml collection plate. • Transfer the lysate mixture to the **DNA Binding Plate**. • Centrifuge for 5 minutes at 3,000 x g.
Step 4 Wash	• Add **300 µl of W1 Buffer** to each well of the **DNA Binding Plate**. • Centrifuge for 5 minutes at 3,000 x g. • Add **600 µl of Wash Buffer** (ethanol added) to each well of the **DNA Binding Plate** to wash again. • Centrifuge for 5 minutes at 3,000 x g. • Discard the flow-through and place the **DNA Binding Plate** back on the 2 ml collection plate. • Centrifuge for 10 minutes at 3,000 x g to remove any ethanol residue.
Step 5 DNA Elution	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (50-100 µl) to increase DNA concentration. • Transfer the **DNA Binding Plate** to a clean **0.35 ml Collection Plate**. • Add **50 µl of preheated Elution Buffer** or TE to the center of each well of the **DNA Binding Plate**. • Let stand for 3 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. • Centrifuge for 5 minutes at 1,000 x g to elute the purified DNA. ❖ For increased efficiency, repeat the elution steps. • Use 5-10 µl of final product for PCR or qPCR (Elute x 1 - 5 µl, Elute x 2 - 10 µl).

96-Well Genomic DNA Kit Blood Spot Protocol

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: centrifugation system for 96-well plates, 2 ml collection plates, absolute ethanol, RNase A (10 mg/ml)

Step 1 Sample Lysis	• Add 3 blood spot pieces to each well of a 2 ml collection plate. • Add 2**00 µl of GT Buffer and 20 µl of Proteinase K** to each well (making sure the sample is completely submerged) and mix by shaking. • Incubate at 60ºC for 30 minutes to lyse the sample. During incubation, shake the collection plate every 5 minutes. • Add 2**00 µl of GB Buffer** to each well and mix vigorously. • Incubate at 70ºC for 20 minutes. During incubation, shake the collection plate every 5 minutes. At this time, preheat the required **Elution Buffer (100 µl per sample)** to 70ºC for DNA Elution step. • Following incubation, centrifuge for 5 minutes at 3,000 x g and transfer the supernatant to a new 2 ml collection plate. • If RNA-free genomic DNA is required, add 5 µl of RNase A to each well and incubate at room temperature for 4 minutes.
Step 2 DNA Binding	• Add 200 µl of absolute ethanol to each sample lysate in the 2 ml collection plate from the previous step. • Mix immediately by pipetting 5-10 times. • Place a **DNA Binding Plate** on a new 2 ml collection plate. • Transfer the lysate mixture to the **DNA Binding Plate**. • Centrifuge for 5 minutes at 3,000 x g.
Step 4 Wash	• Add **300 µl of W1 Buffer** to each well of the **DNA Binding Plate**. • Centrifuge for 5 minutes at 3,000 x g. • Add **600 µl of Wash Buffer** (ethanol added) to each well of the **DNA Binding Plate** to wash again. • Centrifuge for 5 minutes at 3,000 x g. • Discard the flow-through and place the **DNA Binding Plate** back on the 2 ml collection plate. • Centrifuge for 10 minutes at 3,000 x g to remove any ethanol residue.
Step 5 DNA Elution	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (50-100 µl) to increase DNA concentration. • Transfer the **DNA Binding Plate** to a clean **0.35 ml Collection Plate**. • Add **50 µl of preheated Elution Buffer** or TE to the center of each well of the **DNA Binding Plate**. • Let stand for 3 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. • Centrifuge for 5 minutes at 1,000 x g to elute the purified DNA. ❖ For increased efficiency, repeat the elution steps. • Use 5-10 µl of final product for PCR or qPCR (Elute x 1 - 5 µl, Elute x 2 - 10 µl).

96-Well Genomic DNA Kit Semen Protocol

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: centrifugation system for 96-well plates, 2 ml collection plates, absolute ethanol, RNase A (10 mg/ml)

Step 1 Sample Lysis	• Add 200 µl of Semen and **20 µl of Proteinase K** to each well of a 2 ml collection plate and mix by shaking. • Incubate at 60ºC for 10 minutes to lyse the sample. • Add 2**00 µl of GB Buffer** to each well and mix by shaking. • Incubate at 60ºC for 20 minutes. At this time, preheat the required **Elution Buffer (100 µl per sample)** to 60ºC for DNA Elution step. • Following incubation, centrifuge for 5 minutes at 3,000 x g and transfer the supernatant to a new 2 ml collection plate. • If RNA-free genomic DNA is required, add 5 µl of RNase A to each well and incubate at room temperature for 4 minutes.
Step 2 DNA Binding	• Add 200 µl of absolute ethanol to each sample lysate in the 2 ml collection plate from the previous step. • Mix immediately by pipetting 5-10 times. • Place a **DNA Binding Plate** on a new 2 ml collection plate. • Transfer the lysate mixture to the **DNA Binding Plate**. • Centrifuge for 5 minutes at 3,000 x g.
Step 4 Wash	• Add **300 µl of W1 Buffer** to each well of the **DNA Binding Plate**. • Centrifuge for 5 minutes at 3,000 x g. • Add **600 µl of Wash Buffer** (ethanol added) to each well of the **DNA Binding Plate** to wash again. • Centrifuge for 5 minutes at 3,000 x g.

	• Discard the flow-through and place the **DNA Binding Plate** back on the 2 ml collection plate. • Centrifuge for 10 minutes at 1,000 x g to remove any ethanol residue.
Step 5 DNA Elution	Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (50-100 µl) to increase DNA concentration. • Transfer the **DNA Binding Plate** to a clean **0.35 ml Collection Plate**. • Add **50 µl of preheated Elution Buffer** or TE to the center of each well of the **DNA Binding Plate**. • Let stand for 3 minutes or until the **Elution Buffer** or TE is absorbed by the matrix. • Centrifuge for 5 minutes at 1,000 x g to elute the purified DNA. ❖ For increased efficiency, repeat the elution steps. • Use 5-10 µl of final product for PCR or qPCR (Elute x 1 - 5 µl, Elute x 2 - 10 µl).

Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	**Too much sample was used** • Reduce the sample volume.
Low Yield	**Precipitate was formed at DNA Binding Step** • Reduce the sample material. • Prior to loading the plate, break up the precipitate in the ethanol-added lysate.
	Incorrect DNA Elution Step • Ensure that the Elution Buffer or TE is added to the center of each well of the DNA Binding Plate and is absorbed completely. • Ensure to preheat the Elution Buffer or TE prior to adding to the DNA Binding Plate.
Eluted DNA does not perform well in downstream applications.	**Incomplete DNA Elution** • Elute twice to increase yield.
	Residual ethanol contamination • Following the Wash Step, dry the plate with additional centrifugation or incubate at 60ºC for 5 minutes.