EcoPURE Genomic DNA Kit

50 rxns
Cat No: E1075

Shipping : Ship at ambient temperature.
Storage : Store the Kit between 15°C and 25°C
Store Proteinase K at -20°C
Store RNase A at -20°C

General Information

EcoPURE Genomic DNA Kit is designed as a simple and convenient purification of high quality genomic DNA from various samples including whole blood, cultured cells, frozen or fresh tissues, rodent tails, yeast, gram positive or gram negative bacteria, insects, dried blood spots, and buccal swaps. This kit utilizes chaotropic ions and silica-based membrane technology, eliminating the need for expensive resins, hazardous phenol-chloroform extractions, or time-consuming alcohol precipitation. The standard protocol lasts less than 25 minutes and purified DNA can be used directly in PCR, qPCR, sequencing and enzymatic reactions.

Kit Contents

EcoPURE Resuspension Buffer	(15 ml)
EcoPURE Tissue Lysis Buffer	(15 ml)
EcoPURE Lysis Buffer	(15 ml)
EcoPURE Binding Buffer	(22 ml)
EcoPURE Wash Buffer 1*	(13 ml)
EcoPURE Wash Buffer 2**	(8 ml concentrate)
EcoPURE Elution Buffer	(10 ml)
EcoPURE RNase A#	(lyophilized)
EcoPURE Proteinase K#	(lyophilized)
EcoPURE Columns	(50)
EcoPURE Collection Tubes	(50)

*Add 8.8 ml absolute ethanol
**Add 32 ml absolute ethanol

Reconstitute lyophilized Proteinase K in 1.1 ml Proteinase K Storage Buffer. Reconstitute lyophilized RNase A in 1.1 ml RNase Reconstitution Buffer. Proteinase K and RNase A solutions are stable for 1 year when stored at 4°C. For long-term storage (>1 year) store Proteinase K and RNase A solutions at -20°C.

Protocol for Blood Genomic DNA

Each isolation procedure is suitable for isolation of genomic DNA from 200 µl whole blood sample. If extraction of genomic DNA from higher volumes of whole blood is required, use Red Blood Cell Lysis Buffer (10x, Cat No: RBCLB-10x) of EcoTech Biotechnology to isolate white blood cells from up to 9 ml of whole blood and continue with those cells.

1. Add 200 µl *EcoPURE* Lysis Buffer to each 200 µl whole blood sample. Mix well.
2. Add 20 µl *EcoPURE* RNase A to the mixture from step 1 and mix well. Incubate for 3 minutes at room temperature.
3. Add 20 µl *EcoPURE* Proteinase K to the mixture and mix well. Incubate for 10 minutes at 55°C. Extending incubation time to 30 minutes can help increasing the yield.
4. Add 400 µl *EcoPURE* Binding Buffer and mix well.
5. Insert an *EcoPURE* Column into a Collection Tube and transfer the sample from step 4 to the *EcoPURE* Columns. Centrifuge at maximum speed in a tabletop microcentrifuge 1 minute at room temperature.
6. Discard the flow through and add 400 µl *EcoPURE* Wash Buffer 1 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 1 minute at room temperature.
7. Discard the flow through and add 500 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.
8. Discard the flow through and add 200 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column at maximum speed for 2 minutes to completely remove any residual wash buffer.
9. Transfer the *EcoPURE* Column to a clean 1.5 mL microcentrifuge tube (not included).
10. Add 30-50 µL of *EcoPURE* Elution Buffer to the center of the *EcoPURE* Column membrane and incubate the column at room temperature for 5 minutes.
11. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.
12. Discard the *EcoPURE* Column and store the purified DNA at -20°C.

For further information;
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Protocol for Cell Genomic DNA

Each isolation procedure is suitable for isolation of genomic DNA from 10^4 to 10^6 cells. If extraction of genomic DNA from more cells is required, scale up the amounts of reagents used in the entire protocol proportionally.

1. Trypsinize and centrifuge the cells and resuspend the pellet in 200 µl *EcoPURE* Resuspension Buffer by vortexing or pipetting up and down until no cell clumps remain.

2. Add 200 µl *EcoPURE* Lysis Buffer and mix thoroughly. Add 20 µl of *EcoPURE* RNase A to the mixture. Incubate at room temperature for 3 min.

3. Add 20 µl *EcoPURE* Proteinase K and mix well. Incubate for 10 min at 55°C. Extending incubation time to 30 minutes can help increasing the yield.

4. Add 400 µl *EcoPURE* Binding Buffer and mix well.

5. Insert an *EcoPURE* Column into a Collection Tube and transfer the sample from step 4 to the *EcoPURE* Columns. Centrifuge at maximum speed in a tabletop microcentrifuge 1 minute at room temperature.

6. Discard the flow through and add 400 µl *EcoPURE* Wash Buffer 1 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

7. Discard the flow through and add 500 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

8. Discard the flow through and add 200 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column at maximum speed for 2 minutes to completely remove any residual wash buffer.

9. Transfer the *EcoPURE* Column to a clean 1.5 mL microcentrifuge tube (not included).

10. Add 30-50 µL of *EcoPURE* Elution Buffer to the center of the *EcoPURE* Column membrane and incubate the column at room temperature for 5 minutes.

11. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

12. Discard the *EcoPURE* Column and store the purified DNA at -20°C.

Protocol for Tissue Genomic DNA

Each isolation procedure is suitable for isolation of genomic DNA from up to 25 mg fresh or frozen tissue, 0.6–1.2 cm mouse tail, or 0.6 cm rat tail. Typical DNA yield for fresh or frozen tissue is up to 20 µg and for rodent tail is up to 25 µg. If extraction of genomic DNA from more tissue is required, scale up the amounts of reagents used in the entire protocol proportionally.

1a. Cut the tissue or rodent tail into small pieces to make lysis more efficient.

1b. If available, grind the sample in liquid nitrogen to reduce tissue lysis duration.

2. Transfer the grinded or cut sample into a 1.5 ml microcentrifuge tube. Add 200 µl *EcoPURE* Resuspension Buffer.

3. Add 200 µl *EcoPURE* Tissue Lysis Buffer and mix thoroughly. Add 20 µl *EcoPURE* Proteinase K and mix well. Incubate at 55°C until the tissue samples are completely lysed.

Note: Lysis is commonly complete in 1-3 h for tissues and in 6-8 h for rodent tails. Vortexing the samples in every 15 min during incubation might decrease the lysis duration and increase the DNA yield.

4. Add 20 µl of *EcoPURE* RNase A to the mixture. Incubate at room temperature for 3 minutes.

5. Add 400 µl *EcoPURE* Binding Buffer, then add 200 µl absolute ethanol and mix well.

6. Insert an *EcoPURE* Column into a Collection Tube and transfer the mixture from step 5 to the *EcoPURE* Columns. Centrifuge at maximum speed in a tabletop microcentrifuge 1 minute at room temperature.

7. Discard the flow through and add 400 µl *EcoPURE* Wash Buffer 1 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

8. Discard the flowthrough and add 500 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

9. Discard the flow through and add 200 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column at maximum speed for 2 minutes to completely remove any residual wash buffer.

10. Transfer the *EcoPURE* Column to a clean 1.5 mL microcentrifuge tube (not included).

11. Add 30-50 µL of *EcoPURE* Elution Buffer to the center of the *EcoPURE* Column membrane and incubate the column at room temperature for 5 minutes.

12. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

13. Discard the *EcoPURE* Column and store the purified DNA at -20°C.

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Protocol for Yeast Genomic DNA

Each isolation procedure is suitable for isolation of genomic DNA from up to 10^8 yeast cells. If extraction of genomic DNA from more cells is required, scale up the amounts of reagents used in the entire protocol proportionally.

1. Centrifuge the yeast cells and resuspend the pellet in 200 µl *EcoPURE* Resuspension Buffer by vortexing or pipetting up and down until no cell clumps remain.

2. Add 10 µl lyticase (0.5 mg/ml, not provided) and incubate 30 min at 37°C.

3. Add 200 µl *EcoPURE* Lysis Buffer and mix thoroughly. Add 20 µl of *EcoPURE* RNase A to the mixture. Incubate at room temperature for 3 minutes.

4. Add 20 µl *EcoPURE* Proteinase K and mix well. Incubate for 10 minutes at 55°C. Extending incubation time to 30 minutes can help increasing the yield.

5. Add 400 µl *EcoPURE* Binding Buffer and mix well.

6. Insert an *EcoPURE* Column into a Collection Tube and transfer the sample from step 5 to the *EcoPURE* Columns. Centrifuge at maximum speed in a tabletop microcentrifuge 1 minute at room temperature.

7. Discard the flow through and add 400 µl *EcoPURE* Wash Buffer 1 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

8. Discard the flow through and add 500 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

9. Discard the flow through and add 200 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column at maximum speed for 2 minutes to completely remove any residual wash buffer.

10. Transfer the *EcoPURE* Column to a clean 1.5 mL microcentrifuge tube (not included).

11. Add 30-50 µL of *EcoPURE* Elution Buffer to the center of the *EcoPURE* Column membrane and incubate the column at room temperature for 5 minutes.

12. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

13. Discard the *EcoPURE* Column and store the purified DNA at -20°C.

Protocol for Bacterial Genomic DNA

Each isolation procedure is suitable for isolation of genomic DNA from 1 mL of overnight bacterial culture. If extraction of genomic DNA from higher volumes of bacterial culture is required, scale up the amounts of reagents used in the entire protocol proportionally.

For most gram-positive bacteria, the kit must be used in conjunction with the optional lysozyme enzyme (not provided), to effectively lyse the thick peptidoglycan cell walls.

1. Transfer 1 ml of overnight bacterial culture into a 1.5 ml tube and harvest the bacterial culture by centrifugation at 6000 rpm in a tabletop microcentrifuge for 3 minutes at room temperature. Discard the supernatant using a micropipette.

2. Resuspend the bacterial pellet in 200 µl of the *EcoPURE* Resuspension Buffer by vortexing or pipetting up and down until no cell clumps remain.

3. Add 200 µl *EcoPURE* Lysis Buffer and mix thoroughly.

Optional: Add 20 µl of *EcoPURE* RNase A (not provided) to the mixture. Incubate at room temperature for 3 min.

4. Add 20 µl *EcoPURE* Proteinase K and mix well. Incubate for 10 minutes at 55°C. Extending incubation time to 30 minutes can help increasing the yield.

5. Add 400 µl *EcoPURE* Binding Buffer and mix well.

6. Insert an *EcoPURE* Column into a Collection Tube and transfer the sample from step 5 to the *EcoPURE* Columns. Centrifuge at maximum speed in a tabletop microcentrifuge 1 minute at room temperature.

7. Discard the flow through and add 500 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

8. Discard the flow through and add 200 µl *EcoPURE* Wash Buffer 2 *EcoPURE* Column at maximum speed for 2 minutes to completely remove any residual wash buffer.

9. Transfer the *EcoPURE* Column to a clean 1.5 mL microcentrifuge tube (not included).

10. Add 30-50 µL of *EcoPURE* Elution Buffer to the center of the *EcoPURE* Column membrane and incubate the column at room temperature for 5 minutes.

11. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

12. Discard the *EcoPURE* Column and store the purified DNA at -20°C.

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Protocol for Insect Genomic DNA

Each isolation procedure is suitable for isolation of genomic DNA from 30-50 mg insect. Typical DNA yield for 50 mg insect is up to 20 µg. If extraction of genomic DNA from more sample is required, scale up the amounts of reagents used in the entire protocol proportionally.

1. Grind the sample in liquid nitrogen and transfer the grinded sample into a 1.5 ml microcentrifuge tube. Add 200 µl *EcoPURE* Resuspension Buffer.

2. Add 200 µl *EcoPURE* Tissue Lysis Buffer and mix thoroughly. Add 20 µl *EcoPURE* Proteinase K and mix well. Incubate at 55°C for 3 hours.

Note: Lysis is commonly complete in 1–3 hours. Vortexing the samples in every 15 minutes during incubation might decrease the lysis duration and increase the DNA yield.

3. Centrifuge at maximum speed in a tabletop microcentrifuge 1 minute at room temperature. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

4. Add 20 µl of *EcoPURE* RNase A to the mixture. Incubate at room temperature for 3 minutes.

5. Add 400 µl *EcoPURE* Binding Buffer, then add 200 µl absolute ethanol and mix well.

6. Insert an *EcoPURE* Column into a Collection Tube and transfer the mixture from step 5 to the *EcoPURE* Columns. Centrifuge at maximum speed in a tabletop microcentrifuge 1 minute at room temperature.

7. Discard the flow through and add 400 µl *EcoPURE* Wash Buffer 1 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

8. Discard the flow through and add 500 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

9. Discard the flow through and add 200 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column at maximum speed for 2 minutes to completely remove any residual wash buffer.

10. Transfer the *EcoPURE* Column to a clean 1.5 mL microcentrifuge tube (not included).

11. Add 30-50 µL of *EcoPURE* Elution Buffer to the center of the *EcoPURE* Column membrane and incubate the column at room temperature for 5 minutes.

12. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

13.Discard the *EcoPURE* Column and store the purified DNA at -20°C.

Protocol for Dried Blood Spot Genomic DNA

Each isolation procedure is suitable for isolation of genomic DNA from at least 3 punched-out circles. Each punch (3 mm in diameter) is made with a single-hole paper puncher from a dried blood spot.

1. Put at least 3 punched-out circles from a dried blood spot into a 1.5 ml microcentrifuge tube and add 200 µl *EcoPURE* Resuspension Buffer.

2. Add 200 µl *EcoPURE* Tissue Lysis Buffer. Incubate at 85°C for 10 minutes.

3. Add 20 µl *EcoPURE* Proteinase K to the mixture and mix well by vortexing. Incubate for 10 minutes at 55°C. Extending incubation time to 30 minutes can help increasing the yield.

4. Add 400 µl *EcoPURE* Binding Buffer and mix well by vortexing.

5. Incubate at 70°C for 10 minutes.

6. Add 20 µl *EcoPURE* RNase A to the mixture and mix well. Incubate for 3 minutes at room temperature.

7. Insert an *EcoPURE* Column into a Collection Tube and transfer the sample from step 6 to the *EcoPURE* Columns. Centrifuge at maximum speed in a tabletop microcentrifuge 1 minute at room temperature.

8. Discard the flow through and add 400 µl *EcoPURE* Wash Buffer 1 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 1 minute at room temperature.

9. Discard the flow through and add 500 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

10. Discard the flow through and add 200 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column at maximum speed for 2 minutes to completely remove any residual wash buffer.

11. Transfer the *EcoPURE* Column to a clean 1.5 mL microcentrifuge tube (not included).

12. Add 30-50 µL of *EcoPURE* Elution Buffer to the center of the *EcoPURE* Column membrane and incubate the column at room temperature for 5 minutes.

13. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

14. Discard the *EcoPURE* Column and store the purified DNA at -20°C.

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Protocol for Swaps Genomic DNA
Each isolation procedure is suitable for isolation of genomic DNA from swaps.

1. Using sterile techniques, cut the cotton tip of the freshly prepared swaps or stored swaps at proper conditions and add 200 µl *EcoPURE* Resuspension Buffer.

2. Add 200 µl *EcoPURE* Tissue Lysis Buffer, vortex gently, and incubate for 5 minutes at room temperature. Transfer the supernatant to a 1.5 mL microcentrifuge tube.

3. Add 20 µl *EcoPURE* Proteinase K and mix well. Incubate at 55°C until the cells are completely lysed.

4. Add 20 µl of *EcoPURE* RNase A to the mixture and mix well. Incubate at room temperature for 3 minutes.

5. Add 400 µl *EcoPURE* Binding Buffer, then add 200 µl absolute ethanol and mix well.

6. Insert an *EcoPURE* Column into a Collection Tube and transfer the mixture from step 5 to the *EcoPURE* Columns. Centrifuge at maximum speed in a tabletop microcentrifuge 1 minute at room temperature.

7. Discard the flow through and add 400 µl *EcoPURE* Wash Buffer 1 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

8. Discard the flow through and add 500 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

9. Discard the flow through and add 200 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column at maximum speed for 2 minutes to completely remove any residual wash buffer.

10. Transfer the *EcoPURE* Column to a clean 1.5 mL microcentrifuge tube (not included).

11. Add 30-50 µL of *EcoPURE* Elution Buffer to the center of the *EcoPURE* Column membrane and incubate the column at room temperature for 5 minutes.

12. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

13. Discard the *EcoPURE* Column and store the purified DNA at -20°C.

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