EcoPURE BYF Genomic DNA Kit

50 rxns
Cat No: E1085

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 Bacterial/Yeast/Fungi (BYF) Genomic DNA Kit is designed as a simple and convenient purification of high quality genomic DNA from gram positive or gram negative bacteria, yeast, and fungi. 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 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 amount 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 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 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.

For further information;
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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 minutes 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 Fungi Genomic DNA

Each isolation procedure is suitable for isolation of genomic DNA from 100-150 gr wet weighed mycelia/spores or cell pellets collected from 0.5-3 ml fungi culture. If extraction of genomic DNA from more cells is required, scale up the amounts of reagents used in the entire protocol proportionally.

1. Grind mycelia/spores/cell pellets using a mortar and pestle in liquid nitrogen to get a fine powder.

2. Resuspend the grinded powder in 200 µl *EcoPURE* Resuspension Buffer by vortexing or pipetting up and down.

3. Add 200 µl *EcoPURE* Tissue Lysis Buffer and mix thoroughly. Add 20 µl *EcoPURE* Proteinase K and mix well. Incubate for 30 minutes at 55°C.

Note: *Extending incubation time can help increasing the yield. Vortexing the sample in every 5 min during the incubation might increase the DNA yield.*

4. Centrifuge at maximum in a tabletop microcentrifuge 1 min at room temperature. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

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

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

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

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

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