Transiom™ Bacterial DNA Extraction kit

Cat No: TBAC-50/100/200

Contents

Introduction

Transiom™ Bacterial DNA Extraction Kit is designed for the rapid preparation of genomic DNA from wide varieties of bacterial species. Purification is based on spin column chromatography as the separation matrix. This kit is optimized for DNA purification from both Gram-negative and Gram-positive bacterial cells. The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with downstream applications including PCR, real-time PCR, NGS and southern blot analysis.

Quality Control

Quality of Transiom™ Bacterial DNA Extraction Kit is tested on a lot to lot basic by isolation of DNA from different bacterial cultures. The purified DNA was quantified with Nanodrop 8000 and yield of genomic DNA was up to the 10 µg with the OD 260/OD280 ratio of 1.8-2.0. Preparation time for a single sample is less than 50-60 minutes.

Materials supplied by user:

- Nuclease-free microfuge tubes
- Tris-EDTA Buffer
- Water bath Equilibrated to 70^{o}C.
- Nuclease free Water
- Absolute (96%-100) ethanol

Kit Contents

Product	TBAC-50	TBAC-100	TBAC-200	Storage
Buffer TBA	20.0 ml	40.0 ml	80.0 ml	RT
Buffer TBB	20.0 ml	40.0 ml	80.0 ml	RT
Buffer TW I	15.0 ml	30.0 ml	60.0 ml	RT
TransPure™Spin Column	50 nos.	100 nos.	200 nos.	RT
2 ml collection Tubes	50 nos.	100 nos.	200 nos.	RT
TransPure™ Proteinase K	22 mg	22 mg × 2	88 mg	-20^{0}C
TransPure™ RNAse A	10 mg	20 mg	20 mg × 2	-20^{0}C
TransPure™ Lysozyme	25 mg	25 mg × 2	100 mg	-20^{0}C
Elution Buffer	10.0 ml	15.0 ml	30.0 ml	RT
User manual	1	1	1	-

Before Starting

- Read the entire user manual prior to starting the experiment.
- Carry out all experimental procedure/centrifugation at room temperature.
- Use pre-warm water/elution buffer at 70-80 °C for efficient elution of gDNA.

Important

For 50 Extractions

☑ Dilute **Buffer TW I** with **absolute ethanol** as follows and store at room temperature:

Buffer TW I: Add **60 ml** absolute (96%-100%) ethanol per bottle

☑ Add **1100µl Elution Buffer** in **Proteinase K** containing vial.

☑ Add **1100µl Elution Buffer** in **Lysozyme** containing vial.

☑ Add **550µl Elution Buffer** in **RNAse** containing vial.

For 100 Extractions

☑ Dilute **Buffer TW I** with **absolute ethanol** as follows and store at room temperature:

Buffer TW I: Add **120 ml** absolute (96%-100%) ethanol per bottle

☑ Add **1100µl Elution Buffer** in **Proteinase K** containing vial.

Note: 2 Vials of Proteinase K Provided

☑ Add **1100µl Elution Buffer** in **Lysozyme** containing vial.

Note: 2 Vials of Lysozyme Provided

☑ Add **1100µl Elution Buffer** in **RNAse** containing vial.

For 200 Extractions

☑ Dilute **Buffer TW I** with **absolute ethanol** as follows and store at room temperature:

Buffer TW I: Add **240 ml** absolute (96%-100%) ethanol per bottle

☑ Add **4400µlElution Buffer** in **Proteinase K** containing vial.

☑ Add **4400µlElution Buffer** in **Lysozyme** containing vial.

☑ Add **1100µlElution Buffer** in **RNAse** containing vial.

Note: 2 Vials of RNAse A Provided

Bacterial gDNA Kit Protocol

Step 1: Sample Transfer.

Note: 1. For higher yield of Bacterial gDNA proceed below step in same microfuge tube and make sufficient pellet of bacterial biomass.

2. Repeat the below step for sufficient pellet of bacterial biomass. (Optional)

1. Transfer up to 2 ml overnight grown bacterial culture into a microfuge tube (provided by user). Centrifuge at 12,000 rpm for 2 min.

2. Discard growth medium completely and re-suspend the pellet in 250 µl TE Buffer.

Note: Be careful while discarding the supernatant.

3. Add 20 µl of 25 mg/ml lysozyme solution, mix it properly then incubate at 37°C for 30 min. Centrifuge at 5000 rpm for 5 min and discard the supernatant and leave pellet of bacterial biomass and 50-60 µl residual liquid in the tube.

Step 2: Lysis

Note: 1. To ensure efficient lysis, it is essential that the sample and Buffer TBA are mixed thoroughly to yield a homogeneous solution.

2. Ensure that 20 µl of Proteinase K has been added to Lysis buffer as instructed.

4. Add 20 µl of Proteinase K and 375 µl of Buffer TBA followed by 10 µl RNAse A to the microfuge tube, Resuspend the cell pellet by pipetting.

Note: After Incubation if necessary, briefly centrifuge the Collection Tube (~ 1-2 Sec at ~ 2,000 x g) to remove drops from the lid (short spin only).

Note: Do not centrifuge at a higher g-force/rpm in this step!

5. Incubate at 70°C for 25 minutes either on water bath or dry bath. Briefly invert the tube during incubation.

Step 3: Binding of DNA

6. After incubation add 375 µl of Buffer TBB and 200 µl of 96-100 %ethanol to the lysate. Invert the tube for 20-25 times to mix solution properly.

7. Transfer 650 µl lysate to the DNA spin column, and centrifuge at 12,000 rpm for 2 min. Discard the flow through liquid.

8. Repeat above step, until the entire sample has been processed and retain column for further processing.

Step 4: DNA Washing

Note: Add adequate volume of ethanol in Buffer TW I as mentioned in, important point before procession to isolation

9. Place the column into same collection tube. Add 650µl of Buffer TWI. Centrifuge at 10,000 rpm for 1 min. Discard the flow through.

10. Repeat the above "Step-9"

11. Place empty DNA spin column, with the same collection tube and centrifuge at 10,000 rpm for 2 min. Discard the collection tube.

Step 5: DNA Elution

Note: perform the below step twice 30 µl + 30 µl = 60 µl

12. **Place the column into a new sterile 1.5 ml eppendorf tube, add 30 µl preheated (75°C-80°C) Elution buffer. Incubate at room temperature for 5 min. Centrifuge 10,000 rpm for 1 min to elute pure Bacterial DNA.**

Note: The first elution normally yields 60-70 % of DNA bound. A second elution with another 30 µl buffer will yield another 20 % of the DNA.

Note: Elution volume may vary as per downstream process

13. **Discard the Column, and save elute.**

14. **Do not reuse binding columns or collection tubes.**

Trouble Shooting Guide

Problems	Possible reason	Suggestions
Clogged column	Incomplete Lysis	Add the correct volume of Buffer TBA and incubate for specified time at 70°C. It may be necessary to extend incubation time by 10 min.
	Sample is too high	If using more Bacterial culture increase volume of Lysozyme, Proteinase K, RNAse A, Buffer TBA, Buffer TBB and Ethanol. Pass aliquots of lysate through one column successively.
Low DNA Yield	Clogged Column	See above
	Poor elution	Repeat elution or increase elution volume. Incubation of column at 70°C for 5 min with elution buffer may increase yields.
	Improper washing	TWI buffer concentrate must be diluted with ethanol.
Low A260/280 Ratio	Extended centrifugation during elution	Resin from the column may be present in elute and effect the OD absorbance. Avoid centrifugation at speed higher than 12,000 rpm.
	Poor cell lysis due to incomplete mixing with buffer TBA	Repeat the procedure, this time making sure to vortex the sample with Buffer TBA immediately and completely.
No DNA Eluted	Poor Cell Lysis due to improper mixing with Buffer TBA	Mix thoroughly with buffer TBA.

Limited Use and Warranty

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