Transiom Plasmid DNA kit

Cat No: TPLA-50

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Introduction

The Transiom plasmid DNA kit provides a rapid, phenol-free method for purifying plasmid DNA from bacterial cultures. The method employs a modification of the alkaline method of cell lysis and RNAse treatment are used to obtain clear cell lysate with minimal genomic DNA and RNA contaminants. Plasmid DNA purification is simplified with Spin Column technology into three quick steps: Bind, Wash, and Elute. Purified plasmid DNA is immediately ready for a wide variety of downstream applications such as routine screening, restriction enzyme digestion, and DNA sequencing, etc.

Storage and stability:

All components of the Transiom Plasmid DNA kit are stable for at least 12 months at room temperature from date of purchase. RNAse A should be stored at -20°C. During shipment, or storage in cool ambient conditions, precipitates may form. It is possible to dissolve such deposits by warming the solution at 50°C, though we found that they do not inter-fare with overall performance.

Before Starting

- Read carefully all manual instructions before starting.
- RNAse A should be store at -20°C.

Product components and Storage conditions:

Product	TPLA-50	TPLA-100	TPLA-05	Storage
Resuspension Buffer	15 ml	30 ml	1.5 ml (RNAse A Added)	4°C
Rnase A Solution (10 µg/µl)	150 µl	300 µl	Added in Resuspension Buffer	- 20 °C
Lysis Buffer	15 ml	30 ml	1.5 ml	RT
Neutralization Buffer	15 ml	30 ml	1.5 ml	RT
DNA Binding Buffer	20 ml	40 ml	2 ml	RT
****Wash Buffer**	10 ml (add **40 ml** of 96 -100 % ethanol	20 ml (add **80 ml** of 96 -100 % ethanol	1 ml (add **4 ml** of 96 -100 % ethanol	RT
Elution Buffer	10ml	20 ml	1 ml	RT
Low Endotoxin and Nuclease Free Water	10 ml	20 ml	1 ml	RT
TransPure™ Columns	50	100	5	RT
Collection Tubes	50	100	5	RT

Additional Requirement:

- Microcentrifuge Tubes, 96 - 100% Absolute ethanol.

  Note: **To Wash Buffer add 96-100% ethanol (In 1 ml of wash buffer + 4 ml ethanol).

  (In 10 ml of wash buffer + 40 ml ethanol). (In 20 ml of wash buffer + 80 ml ethanol).

- *Add **RNAse A** Solution **150 µl** to **15 ml Resuspension Buffer.**

- *Add **RNAse A** Solution **300 µl** to **30 ml Resuspension Buffer.**

Materials supplied by user:

- Nuclease-free 1.5 ml centrifuge tubes.
- Water bath Equilibrated to 55°C.
- Absolute (96%-100%) ethanol (Equilibrated at room temperature).

Plasmid DNA kit Protocol:

1. **Pipette about 1 ml of *E.coli* cells into a 1.5 ml microfuge/Eppendorf tubes. Centrifuge the sample at 10,000 rpm for 2-5 minutes at room temperature.**

2. **Discard the supernatant, and resuspend the cell pellet in 250 µl of Resuspension Buffer containing RNase A . Mix by tapping gently.**

3. **Add 250 µl of Lysis Buffer to the cell suspension. (Do not vortex)**

4. **Mix the suspension by gently tapping or by inverting the tube up and down 8-10 times.**

5. **Add 250 µl of Neutralization Buffer and mix the solution thoroughly by inverting the tube up and down 8-10 times. (Do not vortex).**

6. **Centrifuge at 12,000 rpm for 10 minutes. Discard the pellet and save the supernatant.**

7. **Add 375 µl of DNA Binding Buffer to the clear supernatant and mix.**

8. **Load 550-600 µl of the mixture on to the DNA spin column, centrifuge for 1 -2 minutes and discard the flow through.**

Note: You can save the remaining half of the lysate and freeze it at -20°C for future use. If you plan to use all of it now, this will probably double the amount of the DNA yield.

9. **Wash the DNA spin column with 400 µl of Wash Buffer. Centrifuge the column for 1-2 minutes. Discard the flow through. Wash one more time.**

Note: Add 96-100 % ethanol to the Wash Buffer (In 20 ml of wash buffer + 80 ml ethanol).

10. **Place the DNA spin column into a clean 1.5 ml Eppendorf tube. Add 50 µl of Elution Buffer or Low Endotoxin and Nuclease Free water for eluting DNA. Incubate for 2 minutes at RT and centrifuge for 1-2 minute.**

11. **Analyze DNA on an agarose gel.**

Transiom Plasmid DNA Kit produce quality and quantity that is superior to that of competitors:

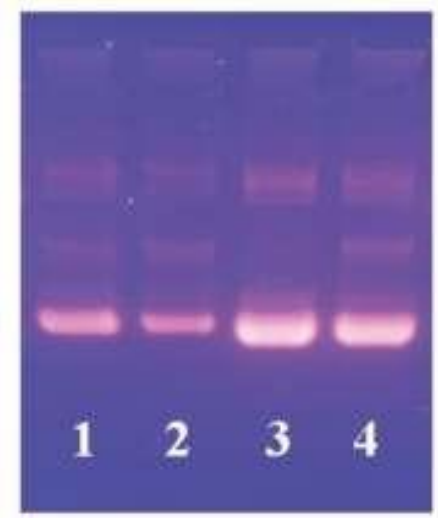

Figure 1 Plasmid DNA Preparation using various kits:

Lanes 1 and 2 represent isolation of DNA from vendors A and B kits respectively;

lanes 3 and 4 represent the isolation of DNA using Transiom Plasmid DNA kit

Trouble shooting guide:

Problem	Possible Cause	Suggestions
Low DNA Yield	Not adequate cell lysis	• Reduce the volume of the cultures taken. • Increase the lysis time. • Mix cell suspension to completely disperse in Lysis Buffer.
	Low copy number plasmid	• Increase the culture volume.
	Overgrown or old bacterial culture	• Avoid incubating cultures for more than 16 hours. • Use fresh cultures. • Avoid prolong storage of cultures prior to plasmid isolation. • Do not save cultures grown at 4°C.
	Too few cells in the sample	• Confirm the concentration of antibiotic and measure the cell density visually before processing the plasmid prep.
	Ethanol not added to Wash Buffer	• Add ethanol to a final 80% concentration as mentioned in the protocol.
Contamination of High molecular weight DNA	Ethanol not added to Wash Buffer	• Do not use cells from a culture grown more than 16 hours as it contains degraded DNA.
	Vigorous mixing of cells with Lysis Buffer	• Mix gently with Lysis Buffer.
Poor quality of Plasmid DNA preparation as reflected by low A260/A280 ratio	Plasmid DNA preparation is contaminated with RNA	• Add RNase A to Resuspension Buffer prior to adding to the cells or increase the resuspension time. • Store RNase at 4°C for prolonged storage.
	Overloading of column	• Avoid using excessive volume of the initial cultures.

Limited Use and Warranty

This product is intended for in vitro research use only. Transiom guarantees the performance of all products in the manner described in our product literature. We reserve the right to change, alter, or modify any product to enhance its performance and design. No other warranties of any kind express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Transiom, Transiom sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Transiom, to replace the products, Transiom shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product. This product is warranted to perform as described in its labeling and in Transiom's literature when used in accordance with instructions. If a Transiom product does not meet your expectations, simply call Transiom Technical Support Team.

For technical support or for more product information, please visit our website at www.transiom.net