Transiom™ Gel DNA/PCR Purification kit

Cat No: TGPK-50/100/200

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Introduction

Transiom™ Gel DNA/PCR Purification kit provides a hassle-free method for high yield recovery of pure DNA from Agarose gels. Simply add the specially formulated Gel extraction Buffer to the gel slice containing your DNA sample, let dissolve, and then transfer to the supplied Column. There is no need for organic denaturants or chloroform. Instead, the product utilizes Fast-Spin column technology to yield high-quality DNA in just 20 minutes. DNA purified using Transiom™ Gel DNA/PCR Purification kit is perfectly suited for use in DNA ligation reactions, sequencing, DNA labeling reactions, PCR, etc.

Quality Control:

Transiom™ Gel DNA/PCR Purification kit allows for DNA fragments from 100 bp to 20 kb can be purified using the mini column with over 80-90 % recovery.

Kit Contents:

Product	TGPK-50	TGPK-100	TGPK-200	Storage
Preps	50	100	200	-
Buffer TGE	25 ml	50 ml	100 ml	RT
Buffer TGB	1.0 ml	2.0 ml	4.0 ml	RT
Buffer TGW	12 ml	24 ml	48 ml	RT
Elution Buffer	10 ml	20 ml	40 ml	RT
TransPure™ Spin Columns	50	100	200	RT
Collection tubes	50	100	200	RT
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Before Starting:

- Read carefully all manual instructions before starting

Important:

For 50 Extractions:

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

Buffer TGW: Add 48 ml absolute (99%-100%) ethanol per bottle.

For 100 Extractions:

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

Buffer TGW: Add 96 ml absolute (96%-100%) ethanol per bottle.

For 200 Extractions:

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

Buffer TGW: Add 192 ml absolute (96%-100%) ethanol per bottle.

Materials supplied by user:

- Nuclease-free microfuge tubes
- Water bath Equilibrated to 60^{o}C.
- Nuclease free ddH_2O
- Absolute (96%-100%) ethanol
- 100% isopropanol

Gel DNA/PCR extraction Kit Protocol

Note: Fresh 1X TAE buffer as running buffer is recommended. Reusing running buffer will result the increase of the pH and then reduce yield and carry over contamination of DNA.

1. Excise the DNA fragment from the Agarose gel with a clean, sharp scalpel. Minimize the size of the gel slice by removing extra Agarose.

2. Weigh the gel slice and transfer into a 2.0 ml Microfuge tube. Add 4.5 volumes of Buffer TGE to 1 volume of gel (i.e. add 450 µl of Buffer TGE to each 100 mg of gel.).
Note: 1. For example, add 450 µl of Buffer TGE to each 100 mg of gel. For >2% Agarose gels, add 6 volumes of Buffer TGE. The maximum amount of gel slice per TransPure™ Spin column is 200 mg; for gel slices >200 mg, use more than one TransPure™ Spin column.
2. The maximum volume of the column reservoir is 650µl. For sample volumes >650 µl, simply load the remainder and spin again.

3. Incubate at 60°C for 15-20 minutes until the gel slice is completely dissolved. Mix the tube by tapping the bottom every 2 minute till the gel has melted completely.
IMPORTANT: Solubilize Agarose completely. For >2% gels, increase incubation time. If the Agarose not solubilize completely increase the incubation time.

4. After the gel slice has dissolved completely add 18 µl Buffer TGB and Mix it well by vortexing

5. After it add 1 volume of 100% Isopropanol to the sample and mix by pipetting.
Note: For example, if the Agarose gel slice is 100 mg, add 100 µl isopropanol. This step increases the yield of DNA fragments ≤500 bp and ≥ 4 kb.

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

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

8. Add 500 µl Buffer TGW to the column and centrifuge at 12,000 rpm for 1 minute at room temperature. Discard the flow through and insert the column, back to the collection tube.
Note: Ensure that ethanol has been added to Buffer TPW as instructed.

9. Repeat the above"step-8" again.

10. Centrifuge the empty DNA column at 12,000 rpm for 2 minute to dry the ethanol residue in the matrix.

Note: The residual ethanol will be removed more efficiently with the column lid open during centrifugation.

11. Place the column into a clean 1.5 ml microfuge tube and add 15 µl /20µl /25µl /30 µl pre-warmed (70-80°C) Elution Buffer to the center of the column. (Note: Add Elution volume as per initial Extracted DNA/ PCR reaction volume i.e. if you are using 25 µl Extracted DNA/ PCR Mixture for purification you must elute it in 20 µl Elution Buffer). Incubate at room temperature for 5 minute. Centrifuge at 12,000 rpm for 1 minute to elute the DNA/ PCR Product.

12. Reload the eluted DNA/PCR Product solution to the column for a second elution in same TransPure™ spin Column. Incubate at room temperature for 2 minute. Centrifuge at 12,000 rpm for 1 minute to elute the DNA/ PCR Product.

IMPORTANT: Ensure that the elution buffer is dispensed directly onto the center of the TransPure™ column membrane for complete elution of bound DNA.

Note: The first elution normally yields 60-70% of the DNA bound. Reload the eluted DNA solution to the column for a second elution will yield another 20% of the DNA that makes the total yield up to 90%.

Note: Elution volume may vary as per downstream process.

Trouble Shooting Guides:

Problems	Possible reason /Solution
Low DNA Yield	Ensure Agarose is completely dissolved
Low DNA Yield	**Poor elution** (Repeat elution or increase elution volume. Incubation of column at 60°C for 5 min with elution buffer may increase yields.)
Low DNA Yield	Ensure that Fresh 1X TAE used for experiment.
Low DNA Yield	**Improper washing** (Wash buffer concentrate must be diluted with ethanol)

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.co.in