Transiom™ Tissue gDNA Extraction kit

Cat No: TTDK-50/100/200

Contents

Introduction

Transiom™ Tissue gDNA isolation kit is designed for the rapid preparation of genomic DNA from tissue samples. Purification is based on spin column technology. DNA binds Column under optimized salt concentrations and releases the bound DNA under low salt and slightly alkali conditions. The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with downstream applications including PCR, real-time PCR, Long-Range PCR, RFLP analysis used for paternity testing and southern blot analysis, etc.

The Transiom™ Tissue gDNA Kit uses a simple four-step method:

1. Effectively disrupting or homogenizing the starting material to release the DNA.
2. Binding DNA to the TransPure™ Binding Column.
3. Removing impurities with wash solution.
4. Eluting purified DNA.

Storage and Stability

All components of the Transiom™ Tissue kit are stable for at least 12 months at storage condition from date of purchase. Proteinase K and RNAse A should be stored at -20°C. During shipment, or storage in cool ambient conditions, precipitates may form in Buffer TTL, Buffer TBL and Buffer TW. It is possible to dissolve such deposits by warming the solution at 50°C, though we found that they do not interfere with overall performance.

Product components and Storage conditions:

Product	TTDK-50	TTDK-100	TTDK-200	Storage
Preps	50	100	200	-
Buffer TTL	20.0 ml	40.0 ml	80.0 ml	RT
Buffer TBL	17.0 ml	35.0 ml	70.0 ml	RT
Buffer TW	15.0 ml	30.0 ml	60.0 ml	RT
Buffer TW I	10.0 ml	20.0 ml	40.0 ml	RT
Elution Buffer	10.0 ml	15.0 ml	20.0 ml	RT
Proteinase K	33 mg	33 mg × 2 Vials	132 mg	-20^0C
RNAse A	5 mg	10 mg	20 mg	-20^0C
TransPure™ Specialized High Binding capacity Columns	50 nos.	100 nos.	200 nos.	RT
Collection Tubes	50 nos.	100 nos.	200 nos.	RT
User manual	1	1	1	-

Before Starting

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

Important:

For 50 Extractions:

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

Buffer TW: Add 15 ml absolute (96%-100%) ethanol per bottle

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

Buffer TW I: Add 40 ml absolute (96%-100%) ethanol per bottle

☑ Add **1100µlElution Buffer** in **Proteinase K** containing vial.

☑ Add **250 µl Elution Buffer** in **RNAse A** containing vial.

For 100 Extractions:

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

Buffer TW: Add 30 ml absolute (96%-100%) ethanol per bottle

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

Buffer TW I: Add 80 ml absolute (96%-100%) ethanol per bottle

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

Note: 2 Vials of Proteinase K enzyme provided.

☑ Add **500 µl Elution Buffer** in **RNAse A** containing vial.

For 200 Extractions:

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

Buffer TW: Add 15 ml absolute (96%-100%) ethanol per bottle

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

Buffer TW I: Add 40 ml absolute (96%-100%) ethanol per bottle

☑ Add **4400 µl Elution Buffer** in **Proteinase K** containing vial.

☑ Add **1000 µl Elution Buffer** in **RNAse A** containing vial

Tissue gDNA Kit Protocol

1. Cut up to 50-100 mg tissue into small pieces (very fine pieces like it looks semi solid liquid), and place in a 2.0 ml micro centrifuge tube. Add 350 µl Buffer TTL.

Note: We strongly recommend to cut the tissue into small pieces to enable more efficient lysis. If desired, lysis time can be reduced by grinding the sample in liquid nitrogen before addition of Buffer TTL and proteinase K.

2. Add 20 µl Proteinase K and 5 µl RNAse A. Mix thoroughly by vortexing, and incubate at 65°C until the tissue is completely lysed. Briefly invert the tube during incubation.

Note: Approximate 45-70 minutes or more depend on sample source. Check tissue lysis during the incubation. Pulse Vortexing the tube 2-3 times during incubation for efficient lysis. If tissue is completely lysed go for further step, higher incubation time after lysis of tissue may cause degradation of the DNA.

Note: The quality and quantity of DNA depend upon the age and storage of Tissue samples.

3. Add 300 µl Buffer TBL to the sample, Vortex for 15-20 seconds and incubate at 65°C for 10 min and mix thoroughly by inverting. Then add 200µl ethanol (96–100%), Mixed by inverting a tube 15- 20 times or Pulse Vortexing. Incubate for 1 min at room temperature (18–25 °C). Briefly centrifuge the Microfuge tube (~ 1-2 s at ~ 2,000 x g) to remove drops from the lid (short spin only).

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!

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

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

6. Place the column into the same collection tube. Add 500 µl of Buffer TW. Centrifuge at 12,000 rpm for 1 min. discard the flow through.

7. Place the column into same collection tube. Add 650µl of TW I. Centrifuge at 12,000 rpm for 1 min. discard the flow through.

8. Place empty DNA spin column, into the same collection tube and centrifuge at 12,000 rpm for 2 min.

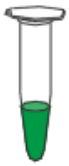
9. Place the column into a new sterile 1.5 ml eppendorf tube, add 30 µl preheated (75°C-80°C) Elution buffer. Incubate at room temperature for 2-3 min. (perform this step twice 30 µl + 30 µl = 60 µl)

10. Centrifuge 10,000 rpm for 1 min to elute pure blood gDNA. 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

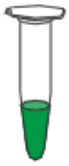
11. Discard the Column, and save elute. Do not reuse binding columns or collection tubes.

FLOW CHART



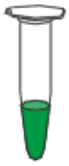
Preparation of samples

- Cut up to 50-100 mg tissue into small pieces(very fine pieces like it looks semi solid liquid)



Lysis

- Add 350µl of TTL and mixed with 20µl of Proteinase K and 5µl of RNAse A



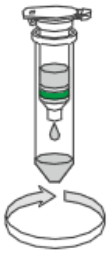
Incubation

- Incubate at 60^0C until tissue is completely lysed

Binding to silica column

- Adjust binding of phase with Buffer TBL and ethanol

Hands on time 40 mins



Washing

- Washing twice with wash buffer TW and TW I
- Dry column

Elution of Pure DNA

Trouble shooting guide

Problems	Possible reason	Suggestions
Clogged column	Incomplete Lysis	Add the correct volume of Buffer TTL and incubate for specified time at 60°C. It may be necessary to extend incubation time.
	Sample is too high	If using more than 100mg of Tissue, increase volume of Proteinase K, RNAse A, Buffer TTL, Buffer TBL and Ethanol. Pass aliquots of lysate through one column successively.
	Sample is too viscous	Divide sample into multiple tubes and adjust the volume to 400 µl with 10mM Tris -HCL.
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	Wash 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 TTL	Repeat the procedure, this time making sure to vortex the sample with Buffer TTL immediately and completely.
No DNA Eluted	Poor Cell Lysis due to improper mixing with Buffer TTL	Mix thoroughly with buffer TTL.
	No Ethanol added to wash buffer concentrate.	Dilute Wash buffer with the indicated volume of absolute ethanol before use.
Eluted Material has Red or Brown Color	Sample volume is too high	Reduce sample volume and Proceed with protocol.

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.

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