Transiom™ Blood gDNA Extraction kit

Cat No: TBDK-50/100/200

Contents

Introduction

The Transiom™ Blood gDNA kit provides a fast, simple technique for preparation of purified and intact DNA from blood. The kit combines the advantages of silica binding with a TransPure™ Spin Column.

The system combines the reversible nucleic acid-binding properties of the matrix with the speed and versatility of spin column technology to yield high quality of gDNA with the OD260/OD280 ratio of 1.8-2.0. Purified DNA is suitable for PCR, restriction digestion, NGS, hybridization techniques and others.

The Transiom™ Blood 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™ Blood gDNA Extraction kit are stable for at least 12 months at storage condition from date of purchase. Proteinase K should be stored at -20°C. During shipment, or storage in cool ambient conditions, precipitates may form in Buffer TBL and Buffer TW. It is possible to dissolve such deposits by warming the solution at 50°C-55°C, though we found that they do not inter-fare with overall performance.

Product components and Storage conditions:

Product	TBDK-50	TBDK-100	TBDK-200	Storage
Preps	50	100	200	-
Buffer TBL	30.0 ml	60.0 ml	120.0 ml	RT
Buffer TW	15.0 ml	30.0 ml	60.0 ml	RT
Buffer TW I	8.0 ml	16.0 ml	32.0 ml	RT
Elution Buffer	10.0 ml	20.0 ml	40.0 ml	RT
Proteinase K	33 mg	33 mg × 02	132 mg	-20°C
TransPure™ Column	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 K 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 32 ml absolute (96%-100%) ethanol per bottle

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

For 100 Extractions

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

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

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

Buffer TW I: Add 64 ml absolute (96%-100%) ethanol per bottle

☑ Add **1100µl Elution Buffer** in **Proteinase K** containing vial **(× 2 Vials)**

For 200 Extractions

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

Buffer TW: Add 60 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 128 ml absolute (96%-100%) ethanol per bottle

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

Blood gDNA Extraction Protocol

1. **Thoroughly mix the blood sample for at least 10 minutes in a rotisserie shaker at room temperature. If the blood has been frozen, thaw completely before mixing for 10 minutes.**

2. **Pipet 20 µl of Proteinase K to sterile 1.5 ml eppendorf tube.**

3. **Add 550 µl of Buffer TBL in eppendorf tube.**

4. **Take 350 µl of fresh blood sample and transfer it to eppendorf tube and Mix thoroughly by vortexing for 30 seconds.**

Note: The quality and quantity of DNA depend upon the age and storage of blood samples. Mix well blood sample through pipetting and then proceed.

5. Incubate at 68°C for 20 minutes either on thermo mixer or water bath or dry bath. Briefly invert the tube 5-7 times during incubation.

Note: To ensure efficient lysis, it is essential that the sample and Buffer TBL are mixed thoroughly to yield a homogeneous solution. The lysate should be blackish red color at this point.

6. After incubation add 350 µl volume of 99-100% ethanol to the lysate. Mixed by inverting a tube 15- 20 times or Pulse Vortexing. Incubate for 30 seconds at room temperature (18–25°C).

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

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

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

10. Place the column into same collection tube. Add 650µl of TW I. Centrifuge at 10,000 rpm for 1 min. discard the flow through.

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

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

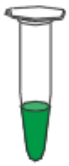
12. Place the column into a new sterile 1.5 ml eppendorf tube, add 30 µl preheated (75°C-80°C) Elution buffer. Incubate at room temperature for 5 min. Centrifuge 10,000 rpm for 1 min to elute pure blood gDNA.

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

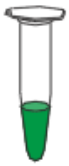
13. Discard the Column, and save elute. Do not reuse binding columns or collection tubes.

FLOW CHART



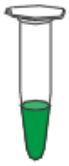
Preparation of samples

- Take 350µl blood sample



Lysis

- Add 550 µl TBL and mixed with 20µl of Proteinase K



Incubation & Phase Separation

- Incubate at 68°C for 20 mins.

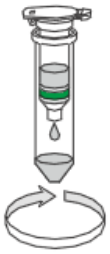
Binding to silica column

- Adjust binding of phase with ethanol

Washing

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

Hands on time 30 mins



Elution of Pure DNA

Trouble shooting guide:

Problems	Possible reason	Suggestions
Clogged column	Incomplete Lysis	Add the correct volume of Buffer TBL and incubate for specified time at 68°C. It may be necessary to extend incubation time by 10 min.
	Sample is too high	If using more than 400µl of blood, increase volume of Proteinase K, 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 TBL	Repeat the procedure, this time making sure to vortex the sample with Buffer TBL immediately and completely.
No DNA Eluted	Poor Cell Lysis due to improper mixing with Buffer TBL	Mix thoroughly with buffer TBL.
	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

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