Transiom™ Sperm DNA Extraction kit

Cat No: TSDK-50/100/200

Contents

Introduction

Transiom™ Sperm DNA kit provides a fast and easy method for isolating DNA from semen samples. 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 DNA with the OD260/OD280 ratio of 1.8-2.0. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques.

Storage and stability:

All components of Transiom™ Sperm DNA kit are stable for at least 12 months at room temperature 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 TSL and TSB. It is possible to dissolve such deposits by warming the solution at 55°C, though we found that they do not inter-fare with overall performance.

Before Starting

- Read carefully all manual instructions before starting.
- Add 1100µl/4400µl Elution Buffer in Proteinase K. (As per pack size of the kit)
- Proteinase K should be store at -20°C.

Product components and Storage conditions:

Product	TDSK-50	TDSK-100	TDSK-200	Storage
Preps	50	100	200	-
Buffer TPW	03.0 ml	06.0 ml	12.0 ml	RT
Buffer TSL	25.0 ml	50.0 ml	100.0 ml	RT
Buffer TSB	20.0 ml	40.0 ml	80.0 ml	RT
Buffer TW I	15.0 ml	30.0 ml	60.0 ml	RT
Elution Buffer	10.0 ml	15.0 ml	30.0 ml	RT
Proteinase K	22 mg	22 mg × 2	88 mg	-20^{0}C
TransPure™ Column	50 nos.	100 nos.	200 nos.	RT
Collection Tubes	50 nos.	100 nos.	200 nos.	RT
User manual	1	1	1	-

Important:

For 50 Extractions

☑ Dilute **Buffer TPW** with **Nuclease Free Water/ Deionized Autoclave water** as follows and store at room temperature:

Buffer TPW: Add 27 ml **Nuclease Free Water/ Deionized Autoclave water** per bottle

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

For 100 Extractions

☑ Dilute **Buffer TPW** with **Nuclease Free Water/ Deionized Autoclave water** as follows and store at room temperature:

Buffer TPW: Add 54 ml **Nuclease Free Water/ Deionized Autoclave water** per bottle

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

Note: 2 Vials of Proteinase K provided.

For 200 Extractions

☑ Dilute **Buffer TPW** with **Nuclease Free Water/ Deionized Autoclave water** as follows and store at room temperature:

Buffer TPW: Add 108 ml **Nuclease Free Water/ Deionized Autoclave water** per bottle

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

Materials supplied by user:

- Nuclease-free 1.5/2.0 ml centrifuge tubes.
- Water bath or Dry Bath Equilibrated to 70°C.
- Nuclease free Water/ Deionized Autoclaved Water
- Absolute (96%-100%) ethanol.

This protocol is optimized for isolating DNA from 100-250 µl fresh or frozen semen samples.

Sperm DNA Extraction Protocol:

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

1. **Take 100-250µl sperm sample and transfer it to 1.5 ml microfuge tube.**

Note: Add adequate volume of Nuclease Free Water/Deionized autoclaved water as mentioned in Buffer TPW.

2. **Add 0.5 ml Buffer TPW to the microfuge tube, Vortex for 10 seconds at full speed.**
3. **Centrifuge at 5000 rpm for 5 minute.**
4. **Carefully remove the supernatant, leaving the pellet.**

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

5. **Re-suspend the pellet in 450 µl Buffer TSL and mix it well by vortexing for 15 seconds.**

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

6. **Add 20 µl of Proteinase K (Provided) and incubate for 15-20 minutes at 70°C. Mix lysate after interval of 5 minutes by inverting the tube.**

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!

7. **After incubation add 350µl volume of TSB add 250µl volume of 99-100% ethanol to the lysate. 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).**

8. **Transfer 650µl lysate to the DNA spin column, and centrifuge at 10,000 rpm for 1 mins. Discard the flow through liquid.**

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

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. **Repeat the above "Step-11" for removal of impurities.**

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

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

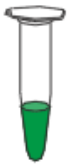
13. **Place the column into a new sterile 1.5 ml eppendorf tube, add 30 µl preheated (75°C-80°C) Elution buffer. Incubate at room temperature for 5 min. Centrifuge 10,000 rpm for 1 min to elute pure Sperm DNA.**

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

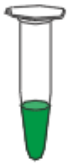
14. **Discard the Column, and save elute. Do not reuse binding columns or collection tubes.**

FLOW CHART



Preparation of samples

- Take 100-250µl Semen sample.



Lysis

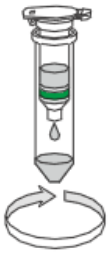
- Add 450µl Buffer TSL and mixed with
- 20µl of Proteinase K

Incubation & Phase Separation

- Incubate at 70^0C for 15-20 min

Binding to silica column

- Adjust binding of phase with Buffer TSB and ethanol



Washing

- Washing twice with wash Buffer TW I
- Dry column

Hands on time 40 mins

Elution of Pure DNA

Trouble shooting guide:

Problems	Possible reason	Suggestions
Clogged column	Incomplete Lysis	Add the correct volume of Buffer TSL and incubate for specified time at 70°C. It may be necessary to extend incubation time by 5- 10 min.
	Sample is too high	If using more than 250µl of Sperm, increase volume of Proteinase K, Buffer TSL, Buffer TSB 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	Wash buffer TW I 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 TSL	Repeat the procedure, this time making sure to vortex the sample with Buffer TSL immediately and completely.
No DNA Eluted	Poor Cell Lysis due to improper mixing with Buffer TSL	Mix thoroughly with buffer TSL.
	No Ethanol added to wash buffer concentrate.	Dilute Wash Buffer TW I with the indicated volume of absolute ethanol before use.

Limited Use and Warranty

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