Transiom™ Soil DNA Extraction kit

Cat No: TSOD-50/100/200

Contents

Introduction:

Transiom™ Soil DNA Extraction Kit comprises a novel and proprietary method for isolating microbial genomic DNA from environmental samples. The kit uses Transiom's Inhibitor Removal Technology® (IRT) and is intended for use with environmental samples containing high humic acid content, including difficult soil types such as compost, sediment, and manure. Other more common soil and stool types have also been used successfully with this kit. Improved IRT combined with more efficient bead beating and lysis chemistry yields high-quality DNA that can be used immediately in downstream applications, including PCR, qPCR, and next-generation sequencing (16S and whole genome).

Principle and procedure:

Transiom Soil DNA Kit is effective at removing PCR inhibitors from even the most difficult soil types. Environmental samples are added to a bead-beating tube for rapid and thorough homogenization. Cell lysis occurs by mechanical and chemical methods. Total genomic DNA is captured on a silica membrane in a spin column format. DNA is then washed and eluted from the membrane and ready for NGS, PCR, and other downstream applications.

The Transiom™ Soil DNA Extraction 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 Buffer.
4. Eluting purified DNA.

Storage and Stability

All components of the Transiom™ Soil DNA 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 TS1, TS2 and TS3. It is possible to dissolve such deposits by warming the buffer at 60°C, though we found that they do not inter-fare with overall performance.

Product components and Storage conditions:

Product	TSOD-50	TSOD-100	TSOD-200	Storage
Preps	50	100	200	-
Buffer SL	45.0 ml	90.0 ml	180.0 ml	RT
Buffer TS1	6.0 ml	12.0 ml	24.0 ml	RT
Buffer TS2	15.0 ml	30.0 ml	60.0 ml	RT
Buffer TS3	15.0 ml	30.0 ml	60.0 ml	RT
Buffer TS4	55.0 ml*	110.0 ml*	220.0 ml*	RT
Buffer TS5	17.0 ml*	34.0 ml*	68.0 ml*	RT
Elution Buffer	10.0 ml	15.0 ml	30.0 ml	RT
Proteinase K	22 mg	22 mg × 2	88 mg	-20^0C
TransBead™ Tube	50 nos.	100 nos.	200 nos.	RT
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 TS4** with **Absolute Isopropanol** as follows and store at room temperature:

Buffer TS4: Add **5.5 ml Absolute** (99%-100%) **Isopropanol** per bottle.

☑ Dilute **Buffer TS5** with **Absolute Ethanol** as follows and store at room temperature:

Buffer TS5: Add **17.0 ml Absolute** (99%-100%) **Ethanol** per bottle.

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

For 100 Extractions:

☑ Dilute **Buffer TS4** with **Absolute Isopropanol** as follows and store at room temperature:

Buffer TS4: Add **11.0 ml Absolute** (99%-100%) **Isopropanol** per bottle.

☑ Dilute **Buffer TS5** with **Absolute Ethanol** as follows and store at room temperature:

Buffer TS5: Add **34.0 ml Absolute** (99%-100%) **Ethanol** per bottle.

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

Note: 2 Vials of Proteinase K Enzyme provided.

For 200 Extractions:

☑ Dilute **Buffer TS4** with **Absolute Isopropanol** as follows and store at room temperature:

Buffer TS4: Add **22.0 ml Absolute** (99%-100%) **Isopropanol** per bottle.

☑ Dilute **Buffer TS5** with **Absolute Ethanol** as follows and store at room temperature:

Buffer TS5: Add **68.0 ml Absolute** (99%-100%) **Ethanol** per bottle.

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

Soil DNA Kit Protocol:

1. **Spin the TransBead™ Tube briefly to ensure that the beads have settled at the bottom. Add up to 350 mg of soil and 800 µl of Buffer SL. Vortex briefly to mix.**

Note: After the sample has been loaded into the TransBead™ Tube, the next step is a homogenization and lysis procedure. The TransBead™ Tube contains a buffer that will (a) help disperse the soil particles, (b) begin to dissolve humic acids, and (c) protect nucleic acids from degradation. Gentle vortexing mixes the components in the TransBead™ Tube and begins to disperse the sample in the buffer.

2. **Add 100 µl of Buffer TS1 and invert several times or vortex briefly.**

Note: Buffer TS1 may be added to the TransBead™ Tube before adding soil sample

3. Vigorously vortex the TransBead™ Tube on a Vortex Mixer at maximum speed for 15-20 min.

Note: If using the Vortex Adapter for more than 12 preps simultaneously, increase the vortexing time by 10-15 min.

Note: Can use any homogenizer for bead beating purpose to lyse the sample.

4. Add 20 µl of Proteinase K and vortex the sample for 20 s and incubate the sample at 70°C for 15 min.

5. Centrifuge TransBead™ Tube at 10,000 rpm for 1 min.

6. Transfer the supernatant to a clean 2 ml Microcentrifuge Tube (Not provided).

Note: Expect 500–800 µl supernatant. The supernatant may still contain some soil particles.

7. Add 270 µl of Buffer TS2 and vortex for 10 s. Incubate at 2-4°C for 5 min.

8. Centrifuge at 10,000 rpm for 1 min. Avoiding the pellet, transfer up to 700 µl of supernatant to a clean 2 ml Microcentrifuge Tube (Not provided).

Note: Expect 500–800 µl supernatant.

9. Add 270 µl of Buffer TS3 and vortex for 10 s. Incubate at 2-4°C for 5 min.

10. Centrifuge tube at 10,000 rpm for 1 min.

11. Avoiding the pellet, transfer up to 800-900µl of supernatant to a clean 2 ml Microcentrifuge Tube.

12. Shake to mix Buffer TS4 and add 1100 µl Buffer TS4 to the supernatant. Vortex for 15s.

13. Load 675 µl sample mixture onto a TransPure™ Spin Column centrifuge at 10,000 rpm for 30s.

14. Discard the flow-through and repeat step 13 to ensure that all of the lysate has passed through the TransPure™ Spin Column.

Note: Repeat step-13 for 3 times to pass all lysate through the TransPure™ Spin Column.

15. Carefully place the TransPure™ Spin Column into a same 2 ml Collection Tube. Avoid splashing any flow-through onto the TransPure™ Spin Column.

16. Add 650 µl of Buffer TS5 to the TransPure™ Spin Column. Centrifuge at 10,000 rpm for 1 min. Discard the flow-through and place the TransPure™ Spin Column into the same Collection Tube.

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

18. Place the column into a new sterile 1.5 ml eppendorf tube, add 25 µl preheated (at 80°C) Elution buffer. Incubate at room temperature for 2 min. (perform this step twice 25 µl + 25 µl = 40 µl)

19. Centrifuge 10,000 rpm for 1 min to elute pure Soil DNA. 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

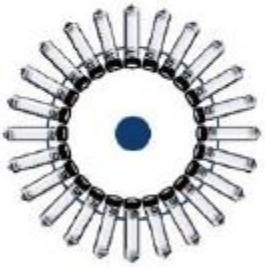
Note: We recommend storing DNA frozen (–20°C to –80°C) as Elution Buffer does not contain EDTA.

Flow Chart

Prepare Sample

Add soil sample to TransBead™ Tube
Add 800 µl Buffer SL and Buffer TS 1

Cell Lysis



Vigorously vortex the TransBead™ Tube on a Vortex Mixer

Hands on time 30 min

Transiom's Inhibitor Removal Technology

Add Buffer TS2
Load into TransPure™ Spin Columns

Add Buffer TS3 in Supernatant
Load into TransPure™ Spin Columns

Bind DNA to TransPure™ Spin Column

Add Buffer TS4 in Supernatant
Load into TransPure™ Spin Columns

Wash

Wash with Buffer TS4
Wash with Buffer TS5

Elute

Elute with Elution Buffer

Trouble shooting guide:

Problems	Possible reason	Suggestions
Poor DNA Recovery	Homogenization was incomplete	Depending on the type of soil, further vortexing with the flat bed vortex or bead beater equipment may be required. However, it is not recommended to increase the vortex time to longer than 15 minutes at maximum speed.
	Inefficient elimination of inhibitory compounds	Repeat with a new sample and add the additional inhibitor removal step.
		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 SL	Repeat the procedure, this time making sure to vortex the sample with Buffer SL and TS1 immediately and completely.
No DNA Eluted	Poor Cell Lysis due to improper mixing with Buffer SL and TS1	Mix thoroughly with Buffer SL & TS1
	No Isopropanol added to TS4 buffer concentrate.	Dilute Buffer TS4 with the indicated volume of absolute Isopropanol before use.
	No Ethanol added to TS5 buffer concentrate.	Dilute Buffer TS5 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.

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