Transiom™ Blood DNA & Viral DNA/RNA Extraction Combo Kit
Cat No: TBVC-50/100/200

Contents

Introduction

With the Transiom™ Blood DNA & Viral DNA/RNA Extraction Combo Kit method Whole EDTA Blood, RNA and DNA viruses are lysed quickly and efficiently by Lysis Buffer TCL. Whole Blood and DNA viruses (e.g., HPV, HBV, etc.) are usually more difficult to lyse and require a digestion with Proteinase K which is supplied in the kit. Lysis buffer and ethanol create appropriate binding conditions of nucleic acids to the TransPure™ Columns. Carrier RNA improves binding and recovery of low-concentrated viral nucleic acids. Contaminations (potential PCR inhibitors) like salts, metabolites, and soluble macromolecular cellular components are removed in simple wash steps with alcoholic buffers TVW and TW1. The nucleic acids are eluted in Elution Buffer and are ready-for-use in subsequent reactions. This Kit is compatible with all types of viral nucleic acid extractions.

Transiom™ Blood DNA & Viral DNA/RNA Extraction Combo Kit uses a simple four-step method:

1. Effectively disrupting or homogenizing the starting material to release the Whole EDTA Blood DNA or Viral RNA/DNA.

2. Binding Blood DNA or Viral RNA/DNA to the TransPure™ Binding Column.

3. Removing impurities with wash solution.

4. Eluting purified Blood DNA or Viral RNA/DNA.

Storage and Stability

All components of the Transiom™ Blood DNA & Viral DNA/RNA Extraction Combo Kit are stable for at least 12 months at storage condition from date of purchase. Proteinase K and Carrier RNA should be stored at -20°C. During shipment, or storage in cool ambient conditions, precipitates may form in Buffer TCL and Buffer TVW. 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	TBVC-50	TBVC-100	TBVC-200	Storage
Preps	50	100	200	-
Buffer TCL	30.0 ml	60.0 ml	120.0 ml	RT
Buffer TVW	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	-20^0C
Carrier RNA (lyophilized)	2.5 mg	5.0 mg	10.0 mg	-20^0C
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 and Carrier RNA should be store at -20°C

Important:

For 50 Extractions:

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

Buffer TVW: Add 15 ml absolute (99%-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 (99%-100%) ethanol per bottle

☑ Add **1100 µL Elution Buffer** in Proteinase K containing vial. (Store at -20°C after reconstitute)

☑ Add **300 µL Elution Buffer** in Carrier RNA containing vial. (Store at -20°C after reconstitute)

For 100 Extractions:

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

Buffer TVW: Add 30 ml absolute (99%-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 (99%-100%) ethanol per bottle

☑ Add **1100 µL Elution Buffer** in Proteinase K containing in each vial. (2 Vials Provided) (Store at -20°C after reconstitute)

☑ Add **600 µL Elution Buffer** in Carrier RNA containing vial. (Store at -20°C after reconstitute)

For 200 Extractions:

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

Buffer TVW: Add 60 ml absolute (99%-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 (99%-100%) ethanol per bottle

☑ Add **4400 µL Elution Buffer** in Proteinase K containing in each vial. (Store at -20°C after reconstitute)

☑ Add **1200 µL Elution Buffer** in Carrier RNA containing vial. (Store at -20°C after reconstitute)

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

3. **Add 550 µl of Buffer TCL 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 20-25 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 TCL 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 650 µl 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 500 µl of Buffer TW. Centrifuge at 12,000 rpm for 1 min. discard the flow through.

10. Place the column into same collection tube. Add 650 µl of TW I. Centrifuge at 10,000 rpm for 30 seconds. Discard the flow through.

11. Place empty DNA spin column, into the same collection tube and centrifuge at 10,000 rpm for 2 min.

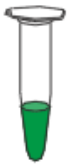
12. Place the column into a new sterile 1.5 ml eppendorf tube, add 30 µl preheated (75°C-80°C) Elution buffer. Incubate at room temperature for 5 min. (perform this step twice 30 µl + 30 µl = 60 µl)

13. 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

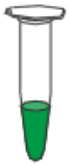
14. Discard the Column, and save elute. Do not reuse binding columns or collection tubes.

FLOW CHART



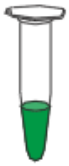
Preparation of samples

- Take 350µl blood sample



Lysis

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



Incubation & Phase Separation

- Incubate at 68^{0}C for 20 mins.

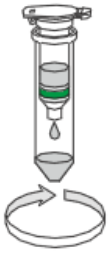
Binding to silica column

- 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

Blood gDNA Extraction Protocol Trouble shooting guide:

Problems	Possible reason	Suggestions
Clogged column	Incomplete Lysis	Add the correct volume of Buffer TCL 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 400 µl of blood, increase volume of Proteinase K, Buffer TCL 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 TCL	Repeat the procedure, this time making sure to vortex the sample with Buffer TCL immediately and completely.
No DNA Eluted	Poor Cell Lysis due to improper mixing with Buffer TCL	Mix thoroughly with buffer TCL.
	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.

Viral RNA/DNA Extraction Protocol

Viral RNA/DNA purification from 500 µL serum, plasma, or cell-free biological fluids

Before starting the preparation:

- **Check if Wash Buffer TVW was prepared according to Before Starting Section.**
- **Check if Carrier RNA and Proteinase K are dissolved in Elution Buffer (stock solution).**
- **The complete procedure should be performed at room temperature (18–25 °C)**

Lyse viruses:

1. Add 20 µL Liquid Proteinase K in a Microfuge tube (1.5 mL, not provided).
Note: Proteinase K may be pipetted into the bottom of the microfuge tube.

2. Add 550 µL Lysis Buffer TCL to the tube.

3. Add 250 µL or 500 µL sample to the tube and mix moderately.
Note: Use sample as per availability. Can use sample from 250µL – 500 µL range.
500 µL sample input can enhance the detection of low viral load in sample.

4. Add 5.0 µL Carrier RNA stock solution to the tube. Mix the tube content by vortexing for 10-15 Sec.

5. Incubate the sample mixture for 15 mins at 70°C.

Adjust binding conditions:

6. Add 450 µL ethanol (99–100 %) to the tube and mix by vortexing (10–15 s).

7. Incubate for 30 seconds at room temperature (18–25 °C).

Bind viral RNA / DNA:

8. Load 650 µl the lysate onto a TransPure™ Spin Column and centrifuge 1 min at 12,000 rpm
Note: Repeat this step, until the entire sample has been processed and retain column for further processing.
Note: If the lysate is not completely drawn through the membrane, repeat the centrifugation at higher g-forces (15,000–20,800 x g for 1 min).

Wash and dry silica membrane:

9. Add 500 µL Wash Buffer TVW to the TransPure™ Spin Column Centrifuge 1 min at 10,000 rpm and discard the flow-through from the collection tube.

10. Add 650 µL Wash Buffer TW I to the TransPure™ Spin Column Centrifuge 30 seconds at 10,000 rpm and discard the flow-through from the collection tube.

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

Elute Viral RNA/DNA:

12. Place the column into a new sterile Nuclease Free 1.5 ml eppendorf tube, add 40 µl Elution buffer. Incubate at room temperature for 2 min.

13. Centrifuge 10,000 rpm for 1 min to elute pure viral RNA/DNA.

14. Keep eluted Viral RNA/DNA on ice or freeze for storage.

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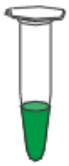
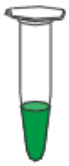


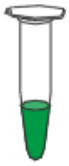
Preparation of samples

- Take 500µl serum, plasma, or cell-free biological fluids



Lysis

- Add 550µl Buffer TCL mixed with 20µl of Proteinase K and 5µl Carrier RNA.



Incubation

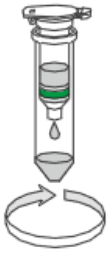
- Incubate at 70°C for 15 min

Binding to silica column

- Adjust binding of phase with ethanol

Washing

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



Elution of Pure Viral RNA/DNA

Hands on time 30 mins

Viral RNA/DNA Extraction Protocol Trouble shooting guide:

Problems	Possible reason	Suggestions
Small amounts or no viral nucleic acids in the eluate	Problems with Carrier RNA	Carrier RNA was not added.
	Viral nucleic acids degraded	• Samples should be processed immediately. Ensure appropriate storage conditions up to the processing. • Check that all buffers have been prepared and stored correctly. If in doubt, use new aliquots of Carrier RNA and RNase-free water.
Problems with subsequent detection	Reduced sensitivity	Change the volume of eluate added to the PCR / RT-PCR.
	Ethanol carry-over	Prolong centrifugation steps in order to remove Buffer TW I completely.
	Carrier RNA interference with detection method	Check if Carrier RNA interferes the detection method. Some detection methods tolerate only limited amounts of carrier RNA
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 TCL	Repeat the procedure, this time making sure to vortex the sample with Buffer TCL immediately and completely.
General Problems	Clogged membrane	Centrifuge plasma lysate before the addition of ethanol and subsequent loading onto the corresponding TransPure™ Spin Columns.

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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