Transiom™ Viral RNA/DNA Extraction kit

Cat No: TVRK-50/100/200

(Sample Size- 500µl)

Contents

Introduction

With the TransPure™ Viral DNA/RNA Extraction method, RNA and DNA viruses are lysed quickly and efficiently by Lysis Buffer TVL. DNA viruses (e.g., HBV, HPV, 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 TW I. The nucleic acids are eluted in Elution Buffer and are ready-for-use in subsequent reactions.

The Transiom™ Viral RNA/DNA Extraction kit uses a simple four-step method:

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

Storage and Stability

All components of the Viral RNA/DNA Extraction 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 TVL and Buffer TVW. 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.

Product components and Storage conditions:

Product	TVRK-50	TVRK-100	TVRK-200	Storage
Preps	50	100	200	-
Buffer TVL	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	15.0 ml	30.0 ml	RT
Proteinase K	22 mg	22 mg × 2 Vials	88 mg	-20^{0}C
Carrier RNA (lyophilized)	2.0 mg	4.0 mg	8.0 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	-

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

☑ Add **400 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 (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 **1100 µL Elution Buffer** in Proteinase K containing vial. (Store at -20°C after reconstitute)

Note: 2 Vials of Proteinase K enzyme provided.

☑ Add **800 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 (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 **4400 µLElution Buffer** in Proteinase K containing vial. (Store at -20°C after reconstitute)

☑ Add **1600 µL Elution Buffer** in Carrier RNA containing vial. (Store at -20°C after reconstitute)

Viral RNA/DNA Extraction Protocol

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

Before starting the preparation:

- **Check if Wash Buffer TVW and TW I were prepared according to Before Starting Section.**
- **Check if Carrier RNA and Proteinase K were 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 (2.0 mL, not provided).
Note: Proteinase K may be pipetted into the bottom of the microfuge tube.

2. Add 550 µl Lysis Buffer TVL to the tube.

3. Add 500 µl sample to the tube and mix moderately.
Note: Use sample as per availability. Can use sample from 300 µL – 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.
Note: Briefly centrifuge the Collection Tube (~1-2 Sec at ~ 2,000 x g) to remove drops from the lid (short spin only).

5. Incubate the sample mixture for 10 min at 70°C.
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).

Adjust binding conditions:

6. Add 450µl ethanol (96–100 %) to the tube and mix by vortexing (10–15 s).

7. 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: Do not centrifuge at a higher g-force/rpm in this step!

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).

9. Repeat the above "Step-8" again.

Wash and dry silica membrane:

10. Add 500 µl Wash Buffer TVW to the TransPure™ Spin Column Centrifuge 1 min at 12,000 rpm and discard the flow-through from the collection tube.

11. Add 650 µl Wash Buffer TW I to the TransPure™ Spin Column Centrifuge 30 seconds at 12,000 rpm and discard the flow-through from the collection tube.

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

Elute Viral RNA/DNA:

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

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

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

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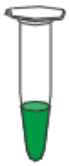
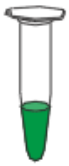


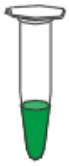
Preparation of samples

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



Lysis

- Add 550µl Buffer TVL mixed with 20µl of Proteinase K and 5.0 µl Carrier RNA.



Incubation

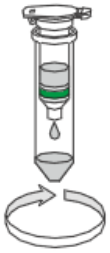
- Incubate at 70°C for 10 min

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

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 TVL	Repeat the procedure, this time making sure to vortex the sample with Buffer TVL immediately and completely.
General Problems	Clogged membrane	Centrifuge plasma lysate before the addition of ethanol and subsequent loading onto the corresponding TransPure™ Spin Columns.