TransMag™ Blood DNA & Viral DNA/RNA Extraction Combo Kit
Cat No: TMBVC-50/100

Contents

Introduction

With the TransMag™ 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 TransMag™ Magnetic Beads. 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 TMB, TW 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.

TransMag™ 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 TransMag™ Magnetic Beads.

3. Removing impurities with wash solution.

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

Storage and Stability

All components of the TransMag™ 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 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	TMBVC-50	TMBVC-100	Storage
Preps	50	100	-
Buffer TCL	25.0 ml	50.0 ml	RT
Buffer TMB	4.5 ml	9.0 ml	RT
Buffer TW	15.0 ml	30.0 ml	RT
Buffer TW I	15.0 ml	30.0 ml	RT
Elution Buffer	10.0 ml	15.0 ml	RT
Proteinase K	22 mg	22 mg × 2	-20°C
Carrier RNA (lyophilized)	2.5 mg	5.0 mg	-20°C
TransMag™ Magnetic Beads	2.7 ml	5.4 ml.	4°C
User manual	1	1	-

Before Starting

- Read carefully all manual instructions before starting
- Proteinase K and Carrier RNA should be store at -20°C
- TransMag™ Magnetic Beads should be store at 4 °C

Important:

For 50 Extractions:

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

Buffer TMB: Add 22 ml absolute (96%-100%) ethanol per bottle

☑ 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 60 ml absolute (96%-100%) ethanol per bottle

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

☑ Add **300 µL Elution Buffer** in Carrier RNA containing vial.

For 100 Extractions:

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

Buffer TMB: Add 44 ml absolute (96%-100%) ethanol per bottle

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

Buffer TW: 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 120 ml absolute (96%-100%) ethanol per bottle

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

☑ Add **600 µL Elution Buffer** in Carrier RNA containing vial.

Note: 2 Vials of Proteinase K enzyme provided.

Blood gDNA Kit Protocol

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.

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

2. **Add 450 µl of Buffer TCL in eppendorf tube.**

3. **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.

4. **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 TCL are mixed thoroughly to yield a homogeneous solution. The lysate should be blackish red color at this point.

5. **After incubation add 450 µl of Buffer TMB and 50 µl of TransMag™ Magnetic Beads to the lysate. Mixed by inverting a tube for 5 min or Mix by shaking at 900~1200 rpm for 3-5 min.**

Note: 1. Before add to sample TransMag™ Magnetic Beads mixed vigorously by vortexing for 30 seconds

2. Buffer TMB and TransMag™ Magnetic Beads can make by premix and then add 500 µl in each sample (Mix Vigorously Before addition into samples)

6. **Place the eppendorf tube on Magnetic Stand and allow beads to separate for 3 minutes. With the eppendorf tube on the Magnetic Stand, perform the aspiration, and then discard the supernatant from the tube.**

Note: Keep the tube in the Magnetic stand. Carefully remove and discard the cleared solution from the tube. Do not touch the beads while removing the solution.

7. **Wash the beads by adding 500 µl Buffer TW resuspend the beads by shaking or mild vortexing for 10 seconds. Briefly short spin the tube to collect the residual sample.**

Note: Do not mix sample vigorously at this step.

8. Place the eppendorf tube on Magnetic Stand and allow beads to separate for 2 minutes. With the eppendorf tube on the Magnetic Stand, perform the aspiration, and then discard the supernatant from the tube.

Note: Keep the tube in the Magnetic stand. Carefully remove and discard the cleared solution from the tube. Do not touch the beads while removing the solution.

9. Wash the beads by adding 650 µl Buffer TW I and resuspend the beads by shaking or mild vortexing for 10-15 seconds. Briefly short spin the tube to collect the residual sample.

Note: Do not mix sample vigorously at this step.

10. Place the eppendorf tube on Magnetic Stand and allow beads to separate for 2 minutes. With the eppendorf tube on the Magnetic Stand, perform the aspiration, and then discard the supernatant from the plate.

Note: Keep the tube in the Magnetic stand. Carefully remove and discard the cleared solution from the tube. Do not touch the beads while removing the solution.

11. Repeat step 10 to Step 11 once.

12. Briefly spin the tube for 5-7 seconds to collect the residual ethanol. Return the tube to the Magnetic Stand for 2 min. Remove the residual ethanol with a 10 µl pipette.

Note: Do not touch the beads while removing the solution.

13. Dry the sample by placing the unsealed tube at Room Temperature for 3-5 minutes or until the residual ethanol completely evaporates.

Note: Do not dry the bead pellet to the point that the pellet appears cracked during any of the bead drying steps in the protocol. Elution efficiency is significantly decreased when the bead pellet is excessively dried.

14. Add 50 µL Elution Buffer. Mix well vigorously on a vortex mixer for 1-1.5 min and briefly spin the tube to collect the liquid.
15. Incubate for 2 minutes at room temperature.

16. Put the tube in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.

17. Remove the cleared supernatant sterile 1.5 ml eppendorf tube.

Blood gDNA Trouble shooting guide

Problems	Possible reason	Suggestions
Low or no recovery	Buffer TMB / Buffer TW did not contain ethanol	Ethanol must be added to Buffer TMB and Buffer TW before used. Repeat procedure with correctly prepare Buffer.
	Low concentration of target DNA in the Sample	Samples were standing at room temperature for too long. Repeated freezing and thawing should be avoided. Low concentration of WBCs in the sample
	Inefficient cell lysis due to insufficient mixing with Buffer TCL	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer TCL immediately and thoroughly by pulse-vortexing.
	Low-percentage ethanol used instead of 100%	Repeat the purification procedure with a new sample. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
A260/A280 ratio for purified nucleic acids is low	Inefficient cell lysis due to insufficient mixing with Buffer TCL	Repeat the procedure with a new sample. Be sure to mix the sample and Buffer TCL immediately and thoroughly by pulse vortexing.
	Inefficient cell lysis due to decreased Proteinase K activity	Repeat the DNA purification procedure with a new sample and with freshly prepared Proteinase K stock solution. Be sure to store the stock solution at -20°C immediately after use. Ensure that Proteinase K is not added directly to Buffer TCL.
A260/A280 ratio for purified nucleic acids is high	High level of residual RNA	In future DNA preparations use the optional RNase step included in the protocols.
DNA does not perform well (e.g. in ligation reaction, Microarray Hybridization, NGS Work)	Salt concentration in eluate too high	Modify the wash step by incubating the sample for 3 min at room temperature after adding 650 µL of Buffer TW I
	Eluate contains residual ethanol	Dry the sample by placing the unsealed tube at Room Temperature for 3-5 minutes or until the residual ethanol completely evaporates
	Inappropriate elution volume used	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly.

Viral RNA/DNA Extraction Protocol:

Viral RNA/DNA purification from 250 µL serum, plasma, or cell-free biological fluids

Before starting the preparation:

- **Check if Wash Buffer TMB, TW, and TW I were 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 10 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 350 µL Lysis Buffer TCL to the tube.

3. Add 250 µL sample to the tube and mix moderately.

4. Add 2.0 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 mins 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. After incubation add 450 µl of Buffer TMB and 20 µl of TransMag™ Magnetic Beads to the lysate. Mixed by inverting a tube for 5 min or Mix by shaking at 900~1200 rpm for 3-5 min.
Note: 1. Before add to sample TransMag™ Magnetic Beads mixed vigorously by vortexing for 30 seconds
2. Buffer TMB and TransMag™ Magnetic Beads can make by premix and then add 500 µl in each sample (Mix Vigorously Before addition into samples)

7. Place the eppendorf tube on Magnetic Stand and allow beads to separate for 2 minutes.

With the eppendorf tube on the Magnetic Stand, perform the aspiration, and then discard the supernatant from the tube.
Note: Keep the tube in the Magnetic stand. Carefully remove and discard the cleared solution from the tube. Do not touch the beads while removing the solution.

8. Wash the beads by adding 500 µl Buffer TW resuspend the beads by shaking or mild vortexing for 10 seconds. Briefly short spin the tube to collect the residual sample.
Note: Do not mix sample vigorously at this step.

9. Place the eppendorf tube on Magnetic Stand and allow beads to separate for 2 minutes. With the eppendorf tube on the Magnetic Stand, perform the aspiration, and then discard the supernatant from the tube.
Note: Keep the tube in the Magnetic stand. Carefully remove and discard the cleared solution from the tube. Do not touch the beads while removing the solution.

10. Wash the beads by adding 650 µl Buffer TW I and resuspend the beads by shaking or mild vortexing for 10-15 seconds. Briefly short spin the tube to collect the residual sample.

Note: Do not mix sample vigorously at this step.

11. Place the eppendorf tube on Magnetic Stand and allow beads to separate for 2-3 minutes. With the eppendorf tube on the Magnetic Stand, perform the aspiration, and then discard the supernatant from the plate.
Note: Keep the tube in the Magnetic stand. Carefully remove and discard the cleared solution from the tube. Do not touch the beads while removing the solution.

12. Briefly spin the tube for 5-7 seconds to collect the residual ethanol. Return the tube to the Magnetic Stand for 2 min. Remove the residual ethanol with a 10 µl pipette.

Note: Do not touch the beads while removing the solution.

13. Dry the sample by placing the unsealed tube at Room Temperature for 3-5 minutes or until the residual ethanol completely evaporates.

Note: Do not dry the bead pellet to the point that the pellet appears cracked during any of the bead drying steps in the protocol. Elution efficiency is significantly decreased when the bead pellet is excessively dried.

14. Add 30 µL Elution Buffer. Mix well vigorously on a vortex mixer for 1-1.5 min and briefly spin the tube to collect the liquid.

15. Incubate for 2 minutes at room temperature.

16. Put the tube in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.

17. Remove the cleared supernatant sterile 1.5 ml eppendorf tube.

Viral RNA/DNA Extraction 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	Dry the sample by placing the unsealed tube at Room Temperature for 3-5 minutes or until the residual ethanol completely evaporates.
	Carrier RNA interference with detection method	Check if Carrier RNA interferes the detection method. Some detection methods tolerate only limited amounts of carrier RNA
	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.
DNA/ RNA does not perform well	Salt concentration in eluate too high	Modify the wash step by incubating the sample for 3 min at room temperature after adding 650 µL of Buffer TW I
	Eluate contains residual ethanol	Dry the sample by placing the unsealed tube at Room Temperature for 3-5 minutes or until the residual ethanol completely evaporates
	Inappropriate elution volume used	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly.

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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