TransMag™ Blood gDNA kit

Cat No: TMBD-50

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Introduction

The TransMag™ Blood gDNA kit provides a fast, simple technique for preparation of purified and intact DNA from blood. TransMag™ Blood gDNA kit is based on the purification method of high binding magnetic particles. The sample is lysed and digested under the action of lysate and Proteinase K enzyme. DNA is released into the lysate. After adding magnetic particles and binding solution, DNA will be adsorbed on the surface of magnetic particles, and impurities such as proteins will be removed without adsorption. The adsorbed particles were washed with washing buffer to remove proteins and impurities, washed with ethanol to remove salts, and finally DNA was eluted by Elution Buffer. Purified DNA is suitable for Next Generation Sequencing, Micro Array, PCR, restriction digestion, hybridization techniques and others.

The TransMag™ Blood gDNA Kit uses a simple four-step method:

1. Effectively disrupting or homogenizing the starting material to release the DNA.
2. Binding DNA to the TransMag™ Magnetic Beads.
3. Removing impurities with wash solution.
4. Eluting purified DNA.

Storage and Stability

All components of the TransMag™ Blood gDNA kit are stable for at least 12 months at storage condition from date of purchase. Proteinase K should be stored at -20°C. TransMag™ Magnetic Beads should store at 4°C. During shipment or storage in cool ambient conditions, precipitates may form in Buffer TBL and Buffer TW. It is possible to dissolve such deposits by warming the solution at 50°C, though we found that they do not inter-fare with overall performance.

Product components and Storage conditions:

Product	TBDK-50	Storage
Preps	50	-
Buffer TBL	25.0 ml	RT
Buffer TMB	4.5 ml	RT
Buffer TW	15.0 ml	RT
Buffer TW I	15.0 ml	RT
Elution Buffer	10.0 ml	RT
Proteinase K	20 mg	-20°C
TransMag™ Magnetic Beads	2.7 ml	4°C
User manual	1	-

Before Starting

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

Important:

☑ 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 **1 ml Elution Buffer** in **Proteinase K** containing vial.

TransMag™ Blood gDNA Kit 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. **Pipet 20 µl of Proteinase K to sterile 1.5 ml eppendorf tube.**

3. **Add 450 µl of Buffer TBL 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 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 TBL are mixed thoroughly to yield a homogeneous solution. The lysate should be blackish red color at this point.

6. **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)

7. 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.

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 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. Repeat step 10 to Step 11 once.

13. 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.

14. 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.

15. 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.
16. Incubate for 2 minutes at room temperature.
17. Put the tube in the magnetic stand and leave for 2 to 3 minutes, until the solution is clear.
18. Remove the cleared supernatant sterile 1.5 ml eppendorf tube.

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 TBL	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer TBL 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 TBL	Repeat the procedure with a new sample. Be sure to mix the sample and Buffer TBL 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 TBL.
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.

Limited Use and Warranty

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