TransMag™ Cell Free DNA Isolation kit

Cat No: TMCF- 48/50/96/100

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Introduction

The TransMag™ Cell Free DNA Isolation Kit designed for purification of high quality Cell Free DNA from cell-free body fluids (such as plasma).The versatile system uses silanol-coated magnetic bead technology to extract and purify circulating cell-free DNA (cfDNA) from mono-nucleosomal and di-nucleosomal complexes from 1mL of plasma. The cell free DNA extraction buffers maximize cell-free DNA (cfDNA) recovery by selecting for and efficiently recovering small DNA fragments from 50 bp - 1000 bp with low elution volumes. TransMag™ Cell Free DNA Isolation Kit protocol is fast and scalable, allowing users to process several samples in one hour, or less. The sample is lysed and digested under the action of lysate and Proteinase K. 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 solution to remove proteins and impurities, washed with ethanol to remove salts, and finally DNA was eluted by Elution Buffer.TransMag™ Cell Free DNA Isolation Kit is a versatile, rapid and cost effective solution for cell free DNA (cfDNA) purification and extraction for downstream PCR or Next Generation Sequencing (NGS) applications, digital PCR, bisulfite sequencing. High yield and sensitivity make the kit well-suited for applications such as cancer diagnosis and monitoring.

The TransMag™ Cell Free DNA Isolation Kit uses a simple four-step method:

1. Effectivelyextract and purify circulating cell-free DNA (cfDNA) from mono-nucleosomal and di-nucleosomal complexes to release the cell-free DNA(cfDNA).

2. Bindingcell-free DNA (cfDNA) to the TransMag™ Magnetic Beads.

3. Removing impurities with wash solution.

4. Eluting purified cell-free DNA (cfDNA).

Storage and Stability

All components of theTransMag™Cell Free DNA Isolation 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. TransMag™ Magnetic Beads should store at 4°C. During shipment or storage in cool ambient conditions, precipitates may form in Buffer TML and Buffer TMW. 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	TMCF-48	TMCF-50	TMCF-96	TMCF-100	Storage
Preps	48	50	96	100	-
Buffer TML	96.0 ml	100.0 ml	192.0 ml	200.0 ml	RT
Buffer TSD	3.0 ml	3.0 ml	6.0 ml	6.0 ml	RT
Buffer TMW	53.0 ml	55.0 ml	106.0 ml	110.0 ml	RT
Buffer TMW I	24.0 ml	25.0 ml	48.0 ml	50.0 ml	RT
Elution Buffer	10.0 ml	10.0 ml	20.0 ml	20.0 ml	RT
Proteinase K	62 mg	66 mg	124 mg	132 mg	-20°C
Proteinase K Dissolve Buffer	2.8 ml	3.0 ml	5.6 ml	6.0 ml	RT
Carrier RNA	2.5 mg	2.5 mg	5.0 mg	5.0 mg	-20°C
TransMag™ Magnetic Beads	2.5 ml	2.75 ml	5.3 ml	5.5 ml	4°C
User manual	1	1	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 store at 4°C

Specially Required items:

- Test Tube Rotator & Mixer (Wavex – LCD Tube Rotator- ABDOS™ or other Test Tube Rotator & Mixer)
- Magnetic Stand for 15 mL tubes. (TransMag™ Magnetic Stand)
- Magnetic Stand for 1.5 ml/ 2.0 ml Microfuge tubes. (TransMag™ Magnetic Stand)

Important:

For 48 Extractions

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

Buffer TMW I: Add **100 ml** absolute (96%-100%) ethanol per bottle

☑ Add **2800 µl Proteinase K Dissolve Buffer** in **Proteinase K** containing vial.

Note: After addition of Proteinase K Dissolve Buffer in Proteinase K containing vial store at -20°C

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

For 50 Extractions

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

Buffer TMW I: Add **100 ml** absolute (96%-100%) ethanol per bottle

☑ Add **3000 µl Proteinase K Dissolve Buffer** in **Proteinase K** containing vial.

Note: After addition of Proteinase K Dissolve Buffer in Proteinase K containing vial store at -20°C

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

For 96 Extractions

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

Buffer TMW I: Add **192 ml** absolute (96%-100%) ethanol per bottle

☑ Add **5600 µl Proteinase K Dissolve Buffer** in **Proteinase K** containing vial.

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

For 100 Extractions

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

Buffer TMW I: Add **200 ml** absolute (96%-100%) ethanol per bottle

☑ Add **6000 µl Proteinase K Dissolve Buffer** in **Proteinase K** containing vial.

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

Recommendation for Plasma Separation and Storage:

To isolate circulating, cell-free nucleic acids from blood samples, we recommend following this protocol which includes a high g-force centrifugation step to remove cellular debris and thereby reduces the amount of cellular or genomic DNA and RNA in the sample.

1. Place Streck™ Cell-Free DNA BCT™ (Recommended) (or other primary blood tubes containing EDTA as anti-coagulant) in centrifuge with swing-out rotor and appropriate buckets.

2. Centrifuge blood samples for 10 min at 1900 x g (3000 rpm) and 4°C temperature setting.

3. Carefully aspirate plasma supernatant without disturbing the buffy coat layer.
About 4–5 ml plasma can be obtained from one 10 ml primary blood tube.
Note: Plasma can be used for circulating nucleic acid extraction at this stage.

4. If plasma will be used for nucleic acid extraction on the same day, store at 2-8°C until further processing. For longer storage, keep plasma frozen at -80°C. Before using the plasma for circulating nucleic acid extraction, thaw plasma tubes at room temperature.
In case of cryoprecipitates, follow these 2 steps:

4a. To remove cryoprecipitates, centrifuge plasma sample for 5 min at 16,000 x g (in fixed angle rotor) and 4°C temperature setting.

4b. Transfer supernatant to new tube and begin with nucleic acid extraction protocol.

Note:
1. It is very much recommended that to perform cell free DNA extraction procedure on same day of Plasma separation Procedure.
2. The quality and quantity of Cell Free DNA depend upon the age and storage of blood samples. Mix well blood sample through pipetting and then proceed.

TransMag™ Cell Free DNA Isolation kit Protocol

1. Transfer 50µl Proteinase K and 1.0 ml Blood Plasma to a new 15 ml sterile centrifuge tube.
Note: The quality and quantity of Cell Free DNA depend upon the age and storage of blood samples.

2. Add 50µl Buffer TSD to the sample and mix well. Incubate at 60°C for 30 min.

Note: To ensure efficient lysis, it is essential that the sample and Buffer TSD are mixed thoroughly to yield a homogeneous solution.

3. Add 1.9 ml Buffer TML, 2µl Carrier RNA and 50µl TransMag™ Magnetic Beads to the sample. Mix upside down (Continuous Inverting) for 10~15 minutes at room temperature OR Mixing by Test Tube Rotator / Mixer (at 40-70 rpm) for 10~15minutes. Then place the tube to the 15 ml capacity Magnetic Stand for 5 minutes until the beads have formed a tight pellet. Then remove the supernatant.
Note: Before add to sample TransMag™ Magnetic Beads mixed homogenously by vortexing for 30 seconds.

4. Add 1000µl Buffer TMW in 15 ml centrifuge tube and vortex for 15-20 seconds to re-suspend beads. Transfer the resuspend solution into new sterile 1.5ml micro centrifuge tube. Place the eppendorf tube on 1.5/2.0 ml capacity Magnetic Stand and allow beads to separate for 3~5 minutes until the beads have formed a tight pellet. With the eppendorf tube on the Magnetic Stand, perform the aspiration, and then discard the supernatant from the tube.
Note: 1. At the time of transfer of resuspend solution ensure that all solution transferred into 1.5 ml microfuge tube. If any remaining it can reduce cfDNA yield.
2. 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.

5. Wash the beads by adding 1000µl Buffer TMW I and resuspend the beads by shaking or mild vortexing for 15 seconds. Briefly short spin the tube to collect the residual sample.

Note: Do not mix sample vigorously at this step.

6. Place the eppendorf tube on the 1.5/2.0 ml capacity Magnetic Stand and allow beads to separate for 3~5 minutes until the beads have formed a tight pellet. 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.

7. Repeat Step 5 and Step 6 once.

8. Briefly spin the tube for 5-7 seconds to collect the residual ethanol. Return the tube to the Magnetic Stand for 2-3 minutes. Remove the residual ethanol with a 10 µl pipette.

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

9. 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.

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

11. Incubate for 3-5 minutes at room temperature.

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

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

14. Store extracted cfDNA at -20°C.

Trouble shooting guide

Problems	Possible reason	Suggestions
Lower yield than expected	Primary blood tube contains an anticoagulant other than EDTA	Anticoagulants may lead to accelerated DNA degradation compared to other than EDTA blood. Repeat the purification procedure with new samples.
	Extended time between blood draw and plasma preparation	Blood cells may disintegrate and release genomic DNA into the plasma, diluting the target nucleic acid.
	Samples frozen and thawed more than once	Repeated freezing and thawing should be avoided as this may lead to DNA degradation. Always use fresh samples or samples thawed only once.
	Low concentration of target DNA in the samples	Samples were left standing at room temperature for too long. Repeat the purification procedure with new samples
	Inefficient sample lysis in Buffer TML	If TransPure™ Proteinase K was subjected to elevated temperature for a prolonged time, it can lose activity. Repeat the procedure using new samples and fresh TransPure™ Proteinase K.
	Buffer TML- Carrier RNA mixture not sufficiently mixed	Mix Buffer TML with Carrier RNA by gently inverting or vortexing the tube of Buffer TML- Carrier RNA at least 10 times.
	Low-percentage ethanol used instead of 96-100%	Repeat the purification procedure with new samples and 96‑100% ethanol. Do not use denatured alcohol, which contains other substances, such as Methanol or Methylethylketone.
	The TransMag™ Magnetic Beads were not properly stored	Remove the TransMag™ Magnetic Beads from the kit and store them at 2-8°C. Do not freeze the beads.
		Allow the beads to warm to room temperature before use.
	An insufficient amount of TransMag™ Magnetic Beads was added	Vortex the tube containing the magnetic beads thoroughly immediately before use.
		If you are preparing a master mix of magnetic beads and TransMag™ Cell Free DNA Lysis/Binding Solution, ensure that the mix is homogeneous before adding sample to the mixture.
	The TransMag™ Cell Free DNA Magnetic Beads are not optimally dried	Drying times may vary depending on the amount of beads used and the environment. Lower volumes of beads require less time to dry. Airflow and humidity in the immediate environment may shorten or lengthen the optimal bead drying time.

Lower yield than expected(continued)		Over dried beads will stick to the wall of the plastics and be difficult to re-suspend.
		Under dried beads may carry ethanol into the eluate and negatively impact downstream applications.
	The sample contains low levels of cfDNA	Increase the starting sample volume.
	Insufficient mixing of the samples with the magnetic beads during the binding step of the manual cfDNA isolation	After adding your sample to the tube containing the TransMag™ Cell Free DNA Lysis/Binding Solution and the magnetic beads, screw tightly the cap of the conical tube, secure the tubes, Microplate Shaker, then shake at speed 7 for the binding step. Alternatively, mix vigorously using a vortex set on maximum speed for 10 minutes using a vortex tube adapter.
DNA or RNA do not perform well in downstream enzymatic reactions	Little or no DNA in the eluate	See “Lower yield than expected” above for possible reasons. Increase the amount of eluate added to the reaction if possible.
	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. The elution volume can be adapted proportionally
	Buffers not mixed thoroughly	Salt and ethanol components of wash Buffer TMW I may have separated out after being left for a long period between runs. Always mix buffers thoroughly before each run.
	New Taq DNA polymerase or PCR chemistry	If enzymes are changed, it may be necessary to used readjust the amount of eluate used for PCR.
	Interference due to carrier RNA	If the presence of carrier RNA in the eluate interferes with the downstream enzymatic reaction, it may be necessary to reduce the amount of carrier RNA or to omit it altogether
Magnetic bead carryover	Loose beads present in the eluate or inadvertently transferred	Be sure to leave the tube on the magnetic stand when removing the eluate containing the cfDNA.
		If beads are carried over into the new tube, place the tube on the magnetic stand again, wait for the beads to pellet and then transfer the sample to another tube.
Abundance of gDNA in eluate	Hemolytic plasma, lipemic plasma, or other	Yields from these types of samples vary greatly from donor to donor. We recommend processing these types of samples using the manual protocol.

	compromised sample types	
Variations in cfDNA yield from donor to donor	Variation in amount of circulating cfDNA. Levels of cfDNA in circulation can range from 1 to 100 ng/mL of plasma or serum depending on the donor.	For samples containing low levels of cfDNA, increase the starting sample volume.

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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