TrusPure Plasma Cell-free DNA Extraction Kit Instructions for Use (Handbook)- Manual/Automatic

For purification and extraction of circulating cell-free DNA and RNA from human plasma and serum

Catalog Numbers: TBRA002, TBRA027
Revision: V1.2
For Research use only

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Contents

Kit Contents and Storage

All components are guaranteed with a shelf life of 18 months from date of manufacture when stored accordingly to the table below. Reagents are compatible with other automated extraction platforms. Please contact info@trustbioo.com for assistance in transitioning to specific automation platforms.

Kit Contents

TrusPure Plasma Cell-free DNA Extraction Kit	Prefilled form	Bottle form
Catalog no.	**TBRA002**	**TBRA027**
Number of preps	**(96 Tests)**	**(50 Tests)**
TrusPure Proteinase K	14.5 ml	7.6 ml
TrusPure Buffer PCL	155 ml	82 ml
TrusPure Buffer PCB *	388 ml	205 ml
TrusPure Buffer Wash II	50 ml	26 ml
TrusPure Buffer Wash A	-	51 ml
TrusPure PS Beads	1.0 ml	0.52 ml
TrusPure PC Beads	-	1.05 ml
TrusPure Buffer Pure S	24 ml	24 ml
TrusPure Buffer Pure E	-	8 ml
Prefilled Reagent plate**	6 pcs	-
8-Tip Comb(2 pcs/bag)	6 bag	-

* If precipitation is present, heat buffer to 37°C until dissolved.

**1. Before loading the plate, please gently tap the plate on the table to ensure no magnetic beads residual on the foil sealed.

2. Suspended magnetic beads won't affect the kit performance.

Storage

TrusPure Plasma Cell-free DNA Extraction Kit should be stored at room temperature upon arrival. All buffer are stable for at least 18 month. If not otherwise stated on the label.

Notes Before Getting Started

- Perform extraction in a clean room.
- Use a new dispensed pipette tip.

Introduction

This kit is designed for extraction of cell-free DNA (cfDNA) from up to 2 ml of plasma/serum. The procedure upon sample loading until completes in about ~55 minutes. Purified plasma DNA can be used for downstream molecular biology applications such as PCR and NGS.

Intended Use

TrusPure Plasma Cell-free DNA Extraction Kit is used for manual sample preparation to isolate circulating cell-free DNA (cfDNA) from plasma samples. The exceptional purity is suitable for PCR and RT-PCR, Genotyping or Sequencing (NGS) assays.

Safety Information and Required Equipment/ Materials Not Provided

- Isopropanol, 100% (for **Part II. Purification of cfDNA** process used)
- Magnetic separator stands to hold 1.5 ml and 50 ml tubes
- DNase decontamination solution.
- DNase free pipette tips and pipettes.
- Note, to avoid the beads residual, a quick spin (such as 1500 rpm for 30 sec) to pellet the beads, and top clear portion can be used for subsequent assays.
- Disposable Plastic consumables (Sterile pipette tips, pipette tips with aerosol barriers are recommended to help prevent cross-contamination)
- Microcentrifuge
- 1.5 ml centrifuge tubes and 50 ml centrifuge tube
- Vortex mixer
- Water bath or heating block capable of holding 50 ml centrifuge tubes at 60°C

Principle and procedure

Sample Storage

Sample Type	Preparation of sample
Plasma or serum samples	Method 1 : 1. Centrifuge the blood samples at 2000 × *g* for 10 minutes. 2. Transfer the plasma to a new centrifuge tube. 3. Centrifuge the plasma samples at 16,000 × *g* for 10 minutes. 4. Transfer the plasma to a new centrifuge tube which can perform the extraction directly. Method 2 : 1. Centrifuged at 6000 × *g* for 30 minutes to remove any residual blood and cell debris. 2. Transfer the plasma to a new centrifuge tube which can perform the extraction directly.

After centrifugation, fresh plasma or serum can be stored at 2–8°C for up to 6 hours. Be careful, for long-term storage, plasma or serum sample need to freeze at –20°C or –80°C in aliquots.

Noted : Frozen plasma or serum samples must not be thawed more than once. Repeated freeze–thawing will affect the performance of purification.

Preparation of sample materials

- Plasma/serum samples is thaw out at room temp or at 4˚C with frequent mixing/rotation and place immediately on ice.
- Set the dry/water bath to 56˚C.
- Centrifuge briefly to collect sample if liquid is seen on caps/tubes.
- Addition of 1 µg carrier RNA to plasma at step 2 is optional.

1. Aliquot desired plasma sample volume (≤2 ml) into a 50 ml tube.

2. According to Reagent volume as Table 1 to add Proteinase K and TrusPure Buffer PCL to pretreat plasma sample.

Table 1. Plasma/serum sample and reagent Volumes

Sample Input	≤2 ml
Proteinase K	0.15 ml
TrusPure Buffer PCL	1.6 ml
Total volume (ml)	≤3.75 ml

3. Vortex at speed ≥7 for 30 sec to mix well. Spin briefly to collect all liquid.
4. Incubate at 56°C for 15 min - 1 hour (Noted: vortex for 30s at speed 3 every 30 mins).
5. After incubation, spin down briefly.
6. Refer TrusPure Buffer PCB and TrusPure PS Beads to **Table 2**, vortex vigorously at speed ≥7 for 30 sec.

Table 2. Sample and reagent Volumes

Sample Lysate	≤3.75 ml
TrusPure PS Beads	50 µl
TrusPure Buffer PCB	4 ml
Total volume (ml)	≤7.8 ml

Note: Before adding TrusPure PS Beads to each sample, to ensure beads resuspend homogeneously.

7. Vortex 50 ml tube vigorously at speed ≥7 for 10 sec to ensure resuspension of beads.
8. Incubate tube with rotation at speed 20 rpm for 15 mins - 1 hour at room temp. Check periodically to ensure beads are in suspension.

Description of procedure-Manual protocol

Part I. cfDNA Extraction from the lysate of plasma

After Preparation of sample materials on Page 5 to Page 6

1. Place tube on the 50 ml magnetic separator for ≥2 mins or until solution is clear of beads.
2. Gently invert tube on magnet three times to collect residual beads from tube walls and cap. Wait for collection the beads for 5 mins on magnetic separator.
   Note: At this time pre-warm TrusPure Buffer Pure E at 56°C.
3. Waste the lysate while the tube sit on the magnet
4. Leave the magnet rack and resuspend beads in 0.5 ml of TrusPure Buffer Wash II. Then transfer beads to a new 1.5 ml tube.
   Note: If residual beads remain in 50 ml tube, use supernatant to resuspend and transfer beads to 1.5 ml tube.
5. Place the 1.5 ml tube on the magnet rack for 30 sec or until solution is clear of beads. Remove and discard supernatant.
6. Add 0.5 ml of TrusPure Buffer Wash A to resuspend beads and vortex at speed ≥7 for 5 sec.
7. Place the tube on magnet for 30 sec and discard supernatant.
8. Repeat **STEP 6 and STEP 7** for a total of 2 washes.
9. Spin down tube to collect residual TrusPure Buffer Wash A, then place the tube to magnet for ~10 sec. Remove and discard the residual buffer.
10. Open the tube to air dry beads for ~15 mins at room temp.
11. Resuspend beads by 50 µl pre-warmed TrusPure Buffer Pure E.
12. Incubate for 5 mins at room temp with vortexing.
13. Spin down tube. And place the tube on magnet for 1 min.
14. Carefully transfer eluted DNA into new 1.5 ml tube

Part II. Purification of cfDNA

Preparation before protocol:

* Keep TrusPure PC Beads to RT for 10-15 mins and pre-warm TrusPure Buffer Pure E to 56°C.

* Isopropanol for **STEP 2**. (*Isopropanol is Materials Not Provided)

1. Add 20 µl TrusPure PC Beads to 50 µl of isolated plasma DNA (the DNA production of **cfDNA Extraction from the lysate of plasma** procedure) in a 1.5 ml tube .Then pipette mix thoroughly.
2. Add 80 µl TrusPure Buffer Pure S and 225 µl of Isopropanol to tube and mix by vortex shaking for 30 mins (speed 7).
   Note: Insufficient shaking will result in lower DNA recovery.
3. Spin down tube and place on the 1.5 ml magnet for ≥10 mins, until the solution is clear. Discard the supernatant slowly to avoid the loss of beads.
4. Keep the tube on the magnet and add 0.5 ml of TrusPure Buffer Wash A.
5. Incubate for 5 mins at room temp and discard supernatant.
6. Repeat **STEP 4 and STEP 5** for two times washing step.
7. After wash step. Open the cap of 1.5 ml tube. Air dry beads on magnet at room temp for 10 mins. Avoid over dry the beads!
8. Add 20-50 µl of pre-warmed TrusPure Buffer Pure E to resuspend beads by pipetting 10 times. Then, incubate for 5 mins at 56°C with intermittent vortexing.
9. Place the tube on the magnet for 5 mins (until solution is clear of beads.)
10. Transfer DNA product to a new 1.5 ml tube slowly (avoid transfer beads to the DNA product). This DNA product is ready for downstream analysis or storage at -20°C.

Description of procedure-Automatic protocol

Preparing reagent plate as the below table or remove the peel sealing foil

of reagent plate.

96 deepwell plate layout of buffer :

Well No	Buffer	Volume
1	TrusPure Buffer Pure E	60 ul
2	**Add Lysate from STEP 4 ***	~500 ul
3	TrusPure Buffer Wash A	300 ul
4	TrusPure Buffer Wash A	300 ul
5	TrusPure PC Beads + TrusPure Buffer Pure S	20 ul+ 380 ul
6	TrusPure Buffer Pure E	50 ul

Part I. cfDNA Extraction from the lysate of plasma

After Preparation of sample materials on Page 5 to Page 6

1. Place tube on the 50 ml magnetic separator for ≥2 mins or until solution is clear of beads.
2. Gently invert tube on magnet three times to collect residual beads from tube walls and cap. Wait for collection the beads for 5 mins on magnetic separator.
3. Waste the lysate while the tube sit on the magnet.
4. Leave the magnet rack and resuspend beads in 0.5 ml of TrusPure Buffer Wash II. Then transfer beads to well 2 and well 8 in 96 deepwell plate. *

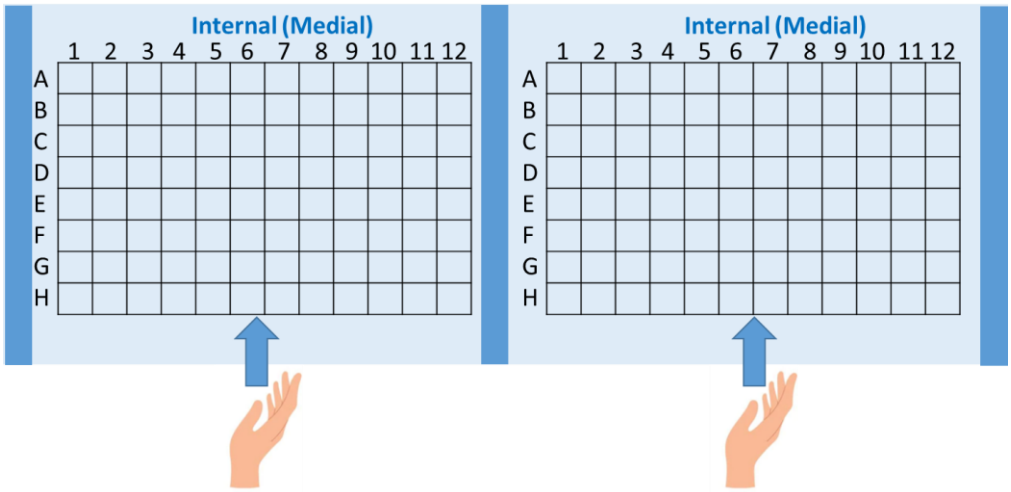
   Note:

   A. If residual beads remain in 50 ml tube, use supernatant to resuspend and transfer beads to 1.5 ml tube.

   B. Before loading the plate, please gently tap the plate on the table to ensure no magnetic beads residual on the foil sealed.

   C .Suspended magnetic beads won't affect the kit performance.

5- Place the reagent **plate** and **8-tip comb** to the instrument.



6- Start the process refer the below table.

Well No	Name	Standby (min)	Mix (min)	Volume	Mix Speed	Mag (sec)	Temp	Pause
2	Wash 1	0	1	400	3	120	0	
3	Wash 2	0	1	300	3	120	0	
1	Elute 1	5	5	100	3	120	0	
2	**Discard**	**0**	**2**	**400**	**3**	**0**	**0**	**V**
5	Purify	0	0	400	0	120	0	
1	Mix	0	8	400	3	120	0	
3	Wash 3	0	1	300	3	120	0	
4	Wash 4	0	1	300	3	120	0	
6	Elute 2	5	5	100	3	120	50	
5	Discard	0	1	400	2	0	0	

Part II. Purification of cfDNA

1. During **PAUSE step** (after ~15mins), need to pick up the reagent plate to add 90 ul of TrusPure Buffer Pure S, 225 ul of Isopropanol to Well 1 and Well 7.
2. After adding the buffer, place the reagent plate back to instrument.
3. Then continue the process.
4. Finish whole process, transfer the extracted nucleic acid product from Well 6 and Well 12 to 1.5 ml tube.

Troubleshooting guide

This troubleshooting guide may be helpful in solving common problem. For more question or information, please contact with TrustBio Technical Service info@trustbioo.com. Our specialist in TrustBio Technical Service will be glad to response your question and please feel free to discuss with us. TrustBio will be always with you.

Lower or no nucleic acids	
Samples frozen and thawed repeatedly	Repeatedly freezing and thawing would lead to DNA degradation. Will suggest to using fresh samples or samples thawed only once before extraction.
Low concentration of DNA in the samples	Samples were thawing at room temperature for long time. Repeat the purification procedure with fresh samples.
No signal in the downstream analysis	Confirm the positive control, No template control and internal control to clarify the possible causes. Readjust the amount of eluate used for PCR.

Document Revision History

Document Revision Information	
Version	**Publish Date**
V1.0	February 2022
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Manufacturer

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