TrusPure Urine Cell-free DNA Extraction Kit Instructions for Use (Handbook)-Manual

For purification and extraction of circulating cell-free DNA and RNA from human urine sample

Catalog Numbers: TBRA028
Revision: V1.2
For Research use only

June 2022

TrustBio Corporation

19F-8., No. 95, Sec. 1, Xintai 5th Rd., Xizhi Dist.,New Taipei City 221416 , Taiwan (R.O.C.)

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 Urine Cell-free DNA Extraction Kit	Bottle form
Catalog no.	**TBRA028**
Number of preps	**(50 Tests)**
TrusPure Proteinase K	7.6 ml
TrusPure Buffer UCL	41 ml
TrusPure Buffer UCB *	210 ml
TrusPure Buffer Wash II	25.5 ml
TrusPure Buffer Wash A	105 ml
TrusPure US Beads	1.26 mlx2
TrusPure UC Beads	2.05 ml
TrusPure Buffer Pure S	3.1 ml
TrusPure Buffer Pure E	8 ml
Prefilled Reagent plate**	-
8-Tip Comb(2 pcs/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 Urine 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 15 ml of urine. The procedure upon sample loading until completes in about ~180 minutes. Extra purified procedure could obtain pure urine cfDNA which can be directly used for downstream molecular biology applications such as PCR and NGS.

Intended Use

TrusPure Urine Cell-free DNA Extraction Kit is used to manually isolate circulating cell-free DNA (cfDNA) from urine 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 **Preparation of sample materials** 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

Suggest to collecting urine after no liquid intake for 2hrs. Otherwise it may risk be too dilute.

Frozen urine samples must not be thawed more than once. Repeated freeze–thawing will affect the performance of purification.

Sample Type	Preparation of sample
Urine sample	1. To preserve the urine DNA, add EDTA buffer to final concentration in 40mM while urine collection. 2. After urine and EDTA are well mixed, it can be kept at 4C for up to 4hrs. But recommend to store at -20C right away for long-term storage. 3. Performing extraction process, can take suitable sample volume to 50ml tube directly. And follow the step in **Preparation of sample materials** as below.

Preparation of sample materials

- Urine 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 urine sample volume into a 50 ml tube.
2. According to Reagent volume as Table 1 to add Proteinase K and TrusPure Buffer UCL to pretreat urine sample in 50 ml centrifuge tube.

Table 1. Urine sample and reagent Volumes

Urine Sample Input	TrusPure Proteinase K (ml)	TrusPure Buffer UCL (ml)	TrusPure Buffer UCB (ml)	Isopropanol (ml)	Total volume (ml)
5 ml	0.15	0.8	4.1	6.7	16.7
10 ml	0.15	1.6	8.1	13.2	33.0
X ml	0.15	0.16 X	0.81X	1.32X	-

*Isopropanol is Materials Not Provided

3. Incubate 50 ml centrifuge tube at 56°C for 1 hour and vortex for 30 sec at speed 3 every 15 mins or use thermal shaker at 1000 rpm.
4. After incubation, centrifuge briefly.
5. According to **Table 1,** add TrusPure Buffer UCB and Isopropanol vortex at speed ≥7 for 30 sec.
6. Add 40 µl TrusPure US Beads to the 50 ml sample tube.

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

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

   Note: <u>Overnight incubation is recommended to obtain maximum yield.</u>

Description of procedure

Part I. cfDNA Extraction from the lysate of urine

1. Place tube on the 50 ml magnet 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 magnet.
   Note: At this time pre-warm TrusPure Buffer Pure E at 56°C.
3. Waste the lysate while the tube sit on the magnetic separator
4. Leave the magnet rack and resuspend beads in 0.5 ml of TrusPure Buffer CW. 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 I 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 I, then place the tube to magnet for ~10 sec. Remove and discard the residual buffer.
10. Open the tube to air dry beads for ~10 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.

1. Add 20 µl TrusPure UC Beads to 50 µl of isolated product (the DNA production of **cfDNA Extraction from the lysate of urine** procedure) in a 1.5 ml tube .Then pipette mix thoroughly.
2. Mix by vortex shaking for 20 mins (speed 4).
3. Spin down and place tube on the 1.5 ml magnet for ≥ 5 mins and until the solution is clear of beads.
4. Carefully transfer the supernatant to a new 1.5 ml tube.
   Note: Do not transfer the beads during this step.
5. Add 60 µl TrusPure Buffer Pure S and 20 µl TrusPure UC Beads to tube containing the supernatant and vortex mix.
6. Add 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.
7. Briefly centrifuge tube and place on 1.5 ml magnet for ≥10 mins or until solution is clear. Remove and discard supernatant.
   NOTE: Slowly remove the supernatant to avoid bead loss.
8. 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.
9. Keep the tube on the magnet and add 0.5 ml of TrusPure Buffer Wash I.
10. Incubate for 5 mins at room temp and discard supernatant.
11. Repeat **STEP 9 and STEP 10** for two times washing step.
12. 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!
13. 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.
14. Place the tube on the magnet for 5 mins (until solution is clear of beads.)
15. 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.

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
V1.1a	May 2022
V1.2	June 2022

Manufacturer

TrustBio Corporation

www.trustbioo.com

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