TrusPure Genomics DNA Extraction Kit

Instructions for Use (Handbook)

For purification and extraction of Genomics DNA from whole blood, serum, cell lines, mammalian tissues, plant , yeast, FFPE (formalin-fixed paraffin-embedded) tissue, bacterial cells and saliva

Catalog Numbers: TBRA003, TBRA029
Revision: V1.1
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 Genomics DNA Extraction Kit	Prefilled form	Bottle form
Catalog no.	**TBRA003**	**TBRA029**
Number of preps	**(96 Tests)**	**(960 Tests)**
TrusPure Buffer Lysis A	-	255 ml x1
TrusPure Buffer Binding C	30 ml x1	350 mlx1
TrusPure Buffer Wash III	-	875 ml x1
TrusPure Buffer Wash A	-	875 ml x2
TrusPure S01 Beads	-	39 ml x1
TrusPure Buffer Pure E	-	150 ml x1
TrusPure Proteinase K (10mg/ml)	1.92ml x1	19.3 mlx1
Prefilled Reagent plate*	6 pcs	-
8-Tip Comb(2 pcs/bag)	6 bag	-

* 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 Genomics 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 purification of Genomics DNA from whole blood, serum, plasma, swab, saliva or other cell-free body fluids. The procedure upon sample loading until completes in about ~20-25 minutes. TrusPure Genomics DNA Extraction Kit can be used for Extraction of Genomics DNA from a broad range of samples. The purification DNA product is 20–50 kb which can be directly used for downstream molecular biology applications such as PCR, restriction enzyme digestion, Southern blotting and qPCR.

Intended Use

TrusPure Genomics DNA Extraction Kit is used for manual sample preparation to isolate Genomics DNA from whole blood, serum, cell lines, mammalian tissues, plant , yeast, FFPE (formalin-fixed paraffin-embedded) tissue, bacterial cells and saliva. The exceptional purity is suitable for PCR, restriction enzyme digestion, Southern blotting and qPCR.

Safety Information and Required Equipment/ Materials Not Provided

- Magnetic stands to hold 1.5 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 with aerosol barriers are recommended to help prevent cross-contamination)
- Microcentrifuge
- 1.5 ml centrifuge tubes
- Vortex mixer
- Water bath or heating block capable of holding 1.5 ml centrifuge tubes at 60°C
- Automatic magnetic pillar device

Principle and procedure

Sample Storage and preparation

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

Noted : Frozen blood samples must not be thawed more than once. Repeated freeze–thawing will affect the yields of Genomics DNA.

Sample Type	Preparation of sample
Blood	● Fresh or frozen whole blood collected in the presence of anti-coagulants such as EDTA or citrate to prevent clotting and DNA degradation. * Don't use heparin tube. This DNA extracted cannot be used for PCR ● Whole blood sample can be stored at 2–4°C for up to 2 weeks. Be careful, for long-term storage, fresh or frozen blood sample need to freeze at –20°C or –80°C in aliquots. ● Typically 200µl of fresh blood is used for DNA Extraction with the yield of 2-10µg. ● Mammalian blood (e.g., human, mouse) : Up to 200µl Non-mammalian (e.g., bird) : 5-10µl
Dried blood spots/ Blood spot on paper	● Dried blood spots on paper e.g., FTAR card. 1- Pick up 2-5 punches (2-3 mm in size) to 1.5ml centrifuge tube. 2- Add 200-400µl of TrusPure buffer PL1 (unsupplied in the kit) * and 20µl of TrusPure Proteinase K to 1.5ml centrifuge tube. 3- Spin down the tube to let the pretreat buffer cover dried blood spots. 4- Incubate the tube at 55°C for 30 minutes to promote protein digestion.

	5- Spin down the tube. And transfer 200µl of supernatant to perform the extraction process. (If less than 200µl, adjust the final sample volume to 200 µl with PBS.)
Cell culture	● Recommend: do not use more than 0.4x10^6 cells. 1- Cell culture cells should be centrifuged to collect cell (5000 rpm or ~2500 x g, 3 min) 2- Remove supernatant. 3- Resuspended in 200µl of PBS or TE buffer. 4- Then aliquot 200µl of lysate to perform the extraction process.
Tissues	● Use 25-30 mg Fresh or frozen tissue (e.g., mammalian tissue) ● Recommend pulverized in liquid nitrogen with mortar and pestle. Then place the powder in a 1.5 ml centrifuge tube. 1- Place the appropriate tissue sample in 1.5 ml centrifuge tube. 2- Add 200-400µl of TrusPure PL1 buffer (unsupplied in the kit) * and 20µl TrusPure Proteinase K in 1.5ml centrifuge tube. (Ensure the tissue is completely immersed in the buffer.) 3- Incubate the tube at 55°C for 1 hour to promote protein digestion until lysis is complete.(For larger tissue pieces, you may perform overnight digestion.) 4- Spin down the lysate and avoid to pick up any particulate materials. 5- Transfer 200µl of lysate to perform the extraction process.
Plant	● Use 50-100 mg of plant tissue. ● Recommend pulverized in liquid nitrogen with mortar and pestle. Then place the powder in a 1.5 ml centrifuge tube. 1- Place the appropriate tissue sample in 1.5 ml centrifuge tube. 2- Add 200-400µl of TrusPure PL3/PL4 buffer (unsupplied in the kit) * in 1.5ml centrifuge tube. (Ensure the plant is completely immersed in the buffer.) 3- Incubate the tube at 55°C for 10 minutes 4- Spin down the lysate. 5- Add 10µl RNase A (unsupplied in the kit) * to the lysate. 6- Incubate at room temperature for 10 minutes. 7- Spin down the lysate and avoid to pick up any particulate materials. 8- Transfer 200µl of lysate to perform the extraction process.

Bacterial cultures	● Gram negative bacteria or Gram positive bacteria :Up to 2 × 109 cells **Gram negative bacteria:** 1- Bacterial culture should be centrifuged (10 min at 5000 x g) to collect bacterial cells. 2- Remove supernatant. 3- Resuspended in 200-400µl of TrsuPure PL2 (unsupplied in the kit)*buffer. 4- Add 20µl TrusPure Proteinase K in 1.5ml centrifuge tube and mix well by vortexing. 5- Incubate the tube at 55°C for 30 minutes 6- Briefly centrifuge to collect any lysate. 7- Add 10µl RNase A (unsupplied in the kit)* to the lysate. 8- Incubate at room temperature for 10 minutes. 9- Spin down the lysate and avoid to pick up any particulate materials. 10- Transfer 200µl of lysate to perform the extraction process. **Gram positive bacteria:** 1- Bacterial culture should be centrifuged (10 min at 5000 x g) to collect bacterial cells. 2- Remove supernatant. **(Optional) Prepare Lysozyme Digestion Buffer (unsupplied in the kit)** or perform the regular homogenization procedures before pretreat process.** a. Resuspended in 200µl of Lysozyme Digestion Buffer. (unsupplied in the kit)** b. Incubate at 37oC for 30 minutes. 3- Resuspended/Add in 200-400µl of TrsuPure PL2 (unsupplied in the kit)*buffer. 4- Add 20µl TrusPure Proteinase K in 1.5ml centrifuge tube and mix well by vortexing. 5- Incubate the tube at 55°C for 30 minutes 6- Briefly centrifuge to collect any lysate. 7- Add 10µl RNase A (unsupplied in the kit)* to the lysate. 8- Incubate at room temperature for 10 minutes. 9- Spin down the lysate and avoid to pick up any particulate materials.

	10- Transfer 200µl of lysate to perform the extraction process.
FFPE (formalin-fixed, paraffin-embedded)	● 20–50 mm^2 : 1-8 sections of <15 µm thick. ● Before extraction FFPE tissue sample, the paraffin must be removed. ● To remove paraffin, using xylene for paraffin extraction. Please also use the appropriate precautions for handling xylene.(Start from step4) 1- Place the appropriate tissue sample in 1.5 ml centrifuge tube. 2- Add 200µl RP Buffer into1.5 ml centrifuge tube and vortex vigorously for 10 seconds. 3- Incubate at 56°C for 3 minutes. 4- Add 200-400µl TrusPure PL1 and 20µl TrusPure Proteinase K in 1.5 ml centrifuge tube. vortex vigorously for a few seconds. 5- Centrifuge at >2,100 × g for 3 minutes at room temperature to pellet the tissue. Carefully remove the supernatant without disturbing the pellet. 6- Incubate at 56℃ until adequate digestion is achieved. 7- Centrifuge the lysate at >2,100 × g for 3 minutes at room temperature to remove any particulate materials. 8- Transfer 200µl the lower blue phase to 1.5 ml centrifuge tube. Then perform the extraction process.

***** TrusPure buffer PL1 /PL2/PL3/PL4, RNase A are the option buffer unsupplied in the kit. Depends on the requirement of customer can purchase from TrustBio.

** Lysozyme Digestion Buffer is optional treatment, is unsupplied in the kit.

Note

Multiple freezing / thawing of the samples should be avoided, since each cycle dramatically diminishes the yield of intact DNA.

If the sample types don't include in the below table, Please contact info@trustbioo.com for assistance the requirement.

Description of procedure

Automatic protocol

1. Peel off sealing aluminum foil of reagent plate or aliquot the buffer as below table.

Well	Buffer Name	Volume(µl)
1/7	TrusPure Buffer Lysis A	400
2/8	TrusPure Buffer Wash III	900
3/9	TrusPure Buffer Wash A TrusPure S01 Beads	900 40
4/10	TrusPure Buffer Wash A	900
5/11	Empty	-
6/12	TrusPure Buffer Pure E	150

1- Add ≤200 µl sample to reagent plate well 1 and well 7.

(If less than 200µl, adjust the final sample volume to 200 µl with PBS.)

2- Aliquot 20 µl TrusPure Proteinase K buffer to well 1 and well 7.

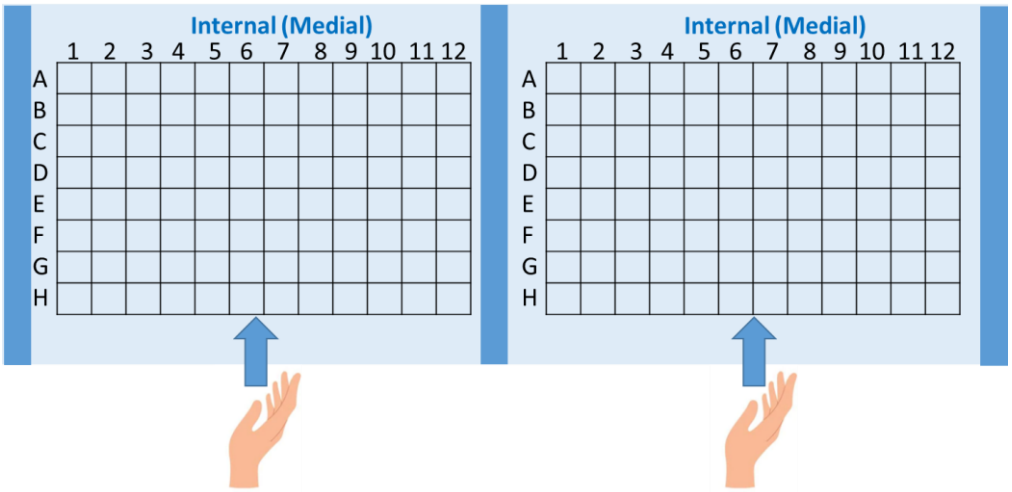
3- Add 300 µl TrusPure Buffer Binding C to well 1 and well 7.

Noted :

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

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

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



5- Start the process as below table.

Step	Well	Name	Standby	Mix (min)	Volume	Mix Speed	Mag (sec)	Temp (℃)
1	3	Transfer	0	1	900	3	30	80
2	1	Lysis	0	20	900	3	30	80
3	2	Wash I	0	3	900	3	30	80
4	3	Wash II	0	2	900	3	30	80
5	4	Wash III	0	1	900	3	30	80
6	6	Elute	5	5	100	3	30	80
7	3	Waste	0	1	900	3	0	0

6- After finishing the process in the instrument. Carefully transfer eluted DNA from well 6 and well 12 into new 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**
V1.0a	May 2022
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Manufacturer

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