TrusPure Viral Nucleic Acids Extraction Kit Instructions for Use (Handbook)-Manual/Automatic

For purification and extraction of viral nucleic acids from serum, plasma, swab, saliva or other cell-free body fluids

Catalog Numbers: TBRA001, TBRA026
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 30 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 Viral Nucleic Acids Extraction Kit	Prefilled form	Bottle form
Catalog no.	**TBRA001**	**TBRA026**
Number of preps	**(96 Tests)**	**(960 Tests)**
TrusPure Buffer LB1*	-	680 ml x1
TrusPure Buffer Wash I	-	875 ml x1
TrusPure Buffer Wash A	-	875 ml x2
TrusPure S Beads	-	12.2 ml x1
TrusPure Buffer Pure E	-	150 ml x1
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 Viral Nucleic Acids Extraction Kit should be stored at room temperature upon arrival. All buffer are stable for at least 30 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 viral nucleic acids from serum, plasma, swab, saliva or other cell-free body fluids. The procedure upon sample loading until completes in about ~20-25 minutes. TrusPure Viral Nucleic Acids Extraction Kit can be used for extraction of viral nucleic acids from a broad range of DNA and RNA viruses. The purification product which can be directly used for downstream molecular biology applications such as PCR or qPCR.

Intended Use

TrusPure Viral Nucleic Acids Extraction Kit is used for manual sample preparation to isolate viral nucleic acids from serum, plasma, swab, saliva or other cell-free body fluids. The exceptional purity is suitable for PCR and RT-PCR.

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

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 yields of viral nucleic acids.

Preparation of sample materials

- Serum/Plasma 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.
- Addition of 10 µl proteinase K (10mg/ml)* and 200 µl PL1 buffer* pretreat 200 µl sample for DNA viral extraction is optional. Use 200 µl sample to mix with 10 µl proteinase K(10mg/ml) and 200 µl PL1 buffer, then incubation at 56°C. After incubation, aliquat 200 µl to perform extraction process.

  ** Proteinase K and PL1 buffer are the option buffer unsupplied in the kit. Depends on the requirement of customer can purchase from TrustBio.*

Sample Type	Preparation of sample
Plasma sample	● 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 ● Do not freeze-thaw the plasma or serum sample more than once.

	● Remove any visible cryoprecipitates from samples. Centrifugation at 7000 × g for 3 minutes. Pick up the clear supernatant for purification. 1. Transfer 200µl of lysate to perform the extraction process.
For Serum, Cerebrospinal fluid (CSF) or Cell free body fluids.	● Remove any visible cryoprecipitates from samples. Centrifugation at 7000 × g for 3 minutes. Pick up the clear supernatant for purification. ● If you are processing <200 µL sample, adjust final volume of the sample to 200 µl using PBS or 0.9% NaCl. 1. Transfer 200µl of lysate to perform the extraction process.
For swab samples	● Dry swab sample (without preservation solution) 1. Add 1ml PBS or 0.9% NaCl to swab tube. 2. To resuspend the sample from swab, vortex and incubate for 10 minutes at room temperature. 3. Transfer 200µl of lysate to perform the extraction process. ● Swab sample storage in liquid preservation solution 1. Vortex the swab tube to make swab infiltrating in the preservation buffer. * ** If you are processing <200 µL sample, adjust final volume of the sample to 200 µl using PBS or 0.9% NaCl.* 2. Transfer 200µl of lysate to perform the extraction process.
For Sputum, BAL or other mucus specimen samples	● If the sample is clear. Adjust final volume of the sample to 200 µl using PBS or 0.9% NaCl. Then start the extraction process directly. ● Liquefaction process for sticky samples.(Option) 1. Use fresh DTT* solution to sample.(final concentration is 0.15%). 2. Incubate sample at room temperature with intermittent mixing until the sample is liquified (up to 30 minutes). 3. Transfer 200µl of lysate to perform the extraction process**. ** DTT solution is unsupplied in the kit, prepare from the user.* ***If you are processing <200 µL sample, adjust final volume of the sample to 200 µl using PBS or 0.9% NaCl.*
For Saliva sample	1. Vortex the swab tube to make swab infiltrating in the preservation buffer. *

	** If you are processing <200 µL sample, adjust final volume of the sample to 200 µl using PBS or 0.9% NaCl.* 2. Transfer 200µl of lysate to perform the extraction process. ● Liquefaction process for sticky samples.(Option) 1. Use fresh DTT* solution to sample.(final concentration is 0.15%). 2. Incubate sample at room temperature with intermittent mixing until the sample is liquified (up to 30 minutes). 3. Transfer 200µl of lysate to perform the extraction process**. ** DTT solution is unsupplied in the kit, prepare from the user.* ***If you are processing <200 µL sample, adjust final volume of the sample to 200 µl using PBS or 0.9% NaCl.*

Description of procedure

Manual protocol

1. Aliquot 200 µl sample volume into a 1.5 ml tube.
2. Add 700 µl TrusPure Buffer LB1 to sample in 1.5 ml centrifuge tube.
3. Vortex at speed 7 for 5 sec.
4. Add 12.5 µl TrusPure S Beads to the 1.5 ml sample tube.
   Note: Before adding TrusPure S Beads to each sample, to ensure beads resuspend homogeneously.
5. Incubate 1.5 ml centrifuge tube at 56°C for 8 min and vortex for 10 sec at speed 3 every 15 sec or use thermal shaker at 1000 rpm.
6. After incubation, spin down the tube for 5 sed.
7. Place tube on the 1.5 ml magnet for ≥10 sec or until solution is clear of beads.
   Note: At this time pre-warm TrusPure Buffer Pure E at 56°C.
8. Waste the lysate while the tube sit on the magnetic separator.
9. Add 0.9 ml TrusPure Buffer Wash I and resuspend the beads.
10. Place the 1.5 ml tube on the magnet rack for 10 sec or until solution is clear of beads. Remove and discard supernatant.

11. Add 0.9 ml of TrusPure Buffer Wash A to resuspend beads and vortex at speed ≥7 for 5 sec.
12. Place the tube on magnetic separator for 10 sec and discard supernatant.
13. Repeat **STEP 11 and STEP 12** for a total of 2 washes.
14. Spin down tube to collect residual TrusPure Buffer Wash A, then place the tube to magnet for ~5 sec. Remove and discard the residual buffer.
15. Open the tube to air dry beads for ~5 mins at room temp.
16. Resuspend beads by 50 µl pre-warmed TrusPure Buffer Pure E.
17. Incubate for 5 mins at room temp with vortexing.
18. Spin down tube. And place the tube on magnet for 30 sec.
19. Carefully transfer eluted DNA into new 1.5 ml tube

Automatic protocol

1. Remove the peel sealing foil of reagent plate or aliquot the buffer as below table.

Well	Buffer Name	Volume(µl)
1/7	TrusPure Buffer LB1	700
2/8	TrusPure Buffer Wash I	900
3/9	TrusPure Buffer Wash A TrusPure S Beads	900 12.5
4/10	TrusPure Buffer Wash A	900
5/11	Empty	-
6/12	TrusPure Buffer Pure E	120

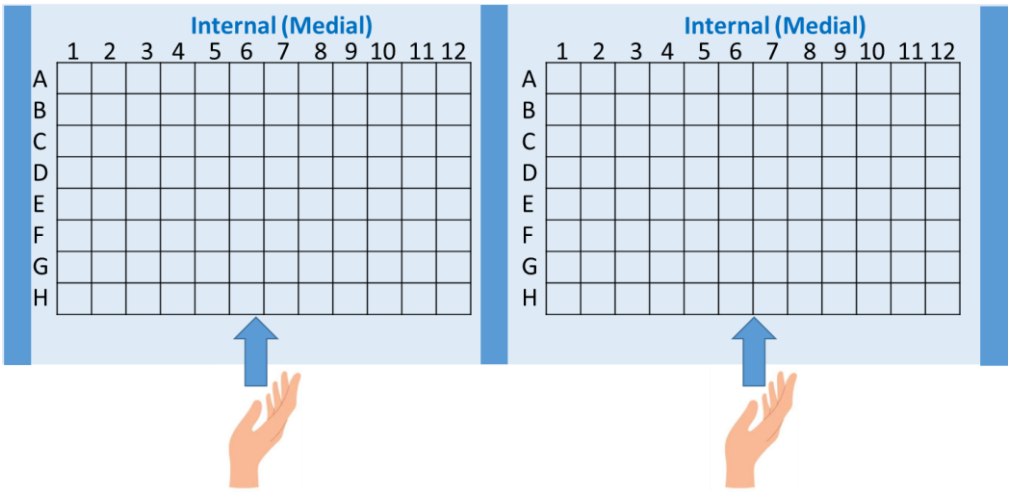
2. Aliquot 200 µl sample volume into well 1 and well 7.

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.

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



4. 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	8	900	3	30	80
3	2	Wash I	0	1	900	3	30	80
4	3	Wash II	0	1	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

5. 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**
V1.0	February 2022
V1.1a	May 2022
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Manufacturer

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