Viral RNA Extraction Kit

REF: KT017

The KREATIVE Viral RNA Extraction Kit is designed to extract the viral RNA from plasma, whole blood, cell-free body fluids (including plasma, serum, urine, CSF and cell culture supernatant), viral stock solution and infected tissues.

For In-Vitro Diagnostic Use Only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KREATIVE TECHNOLABS is strictly prohibited.

Intended Use:
The KREATIVE Viral RNA Extraction Kit is designed to extract viral RNA from plasma, whole blood, cell-free body fluids (including plasma, serum, urine, CSF and cell culture supernatant), viral stock solution and infected tissues samples.

Principle:
This product is suitable for the extraction of various viral RNA from plasma, whole blood, cell-free body fluids (including plasma, serum, urine, CSF and cell culture supernatant), viral stock solution and infected tissues. Compared with the traditional Trizol method, the detection sensitivity can be increased 5-10 times. After the nucleic acid of the dissolved virus is bound to the purified column, the Buffer WBR is washed to remove the residual PCR inhibitor on the purified column, and then eluted with Buffer TE for PCR or RT- PCR reaction.

Components Provided in the Kit:

Component	Size
Nucleic Acid Purification Column	100
2ml Centrifugal Tube	100
Protease K Storage Solution	2.4 ml
Carrier RNA	800 ul
Buffer VL	30 ml
Buffer WBR (concentrate)	6.5ml x 4
Buffer TE	10 ml
Instruction Manual	1

Components Not Provided in the Kit:
1. Anhydrous ethanol
2. 1.5ml centrifuge tube (DNase-free & RNase-free 1.5ml centrifuge tube is recommended)
3. Pipette tips (to avoid contamination in the samples, please select the DNase-free & RNase-free Pipette tips with filter cartridges)
4. Disposable gloves, protective equipment and tissues
5. Desktop small centrifuge (rotor with 1.5ml centrifuge tube and 2ml centrifuge tube)
6. Water Bath and Vortex Shaker
7. Normal saline may be needed

Preparation Before Use:

1. If the centrifuge has refrigeration function, please set the temperature to 25ºC.
2. Set the temperature of the water bath to 56ºC and incubate the **Buffer TE** at 56ºC.
3. Add anhydric ethanol to the **Buffer WBR** according to the instructions on the label of the reagent bottle, and tick the box of the label to mark "ethanol added".
4. According to the number of nucleic acid samples to be prepared, the volume of **Buffer VL** to be used (200 ul Buffer VL / tube) was calculated. It is suggested to increase the volume of **Buffer VL** by 300~500 ul due to possible errors in the infusion process. **Carrier RNA** was added to the ratio of **25 ul Carrier RNA per 1ml Buffer VL volume**, vortex and mix for a few seconds.

Storage Temperature:

1. **Protease K** storage solution and Carrier RNA should be stored at -20ºC.
2. If other reagents and materials are stored at room temperature (15~25ºC), their performance can be kept unchanged for two years; If the product is stored at 2~8ºC, the product life can be extended to more than two years.

Sample Pretreatment:

Plasma, Serum, Cell-free Body Fluids, Viral Stock, Urine specimens, Cerebrospinal Fluid, Herpes fluid, CSF and Cell Culture Supernatant
200 ul of the samples were directly absorbed for the isolation and purification of viral nucleic acid. If the specimen volume is less than 200 ul, PBS solution is added to 200 ul.

** try to isolate and purify viral nucleic acids from fresh isolated or freeze-thaw specimens (with no more than one freeze-thaw cycle).*

Pharyngeal Swab Lotion, Genital Tract Swab Lotion
Add 300 ul pharharyngeal swab lotion, genital tract swab lotion into 1.5 ml centrifuge tube. Centrifuge at 12000 rpm for 5 minutes, and pipette 200 ul of the supernatant for the isolation and purification of viral nucleic acids.

Tissue Lysate of Infected Virus:
Take 10 mg of virus-infected tissue for liquid nitrogen grinding. Add 300 ul PBS solution to the ground tissue and suspend it. 200 ul tissue suspension is used to separate and purify viral nucleic acid.

Stool
1 ml of normal saline is added to the 1.5 ml centrifuge tube. About 200 mg off stool is selected with sterilized toothpick (200 ul is directly absorbed, if the stool is liquid). Add to the 1.5 ml centrifuge tube, and vortex until the stool is completely dispersed. Centrifuge at 12000 rpm for 1 min. Take 200 ul of the supernatant for the isolation and purification of viral nucleic acid.

Procedure:

1. Add **20 ul Protease K Storage Solution** into 1.5 ml centrifuge tube, and then add 200 ul of pretreated sample.
   ** do not add protease K directly to Buffer VL.*

2. Add **200 ul Buffer VL** containing **Carrier RNA** and mix by vortexing for about 15 seconds.

3. Place the centrifuge tube in a 56°C water bath for 10 minutes.

4. Add **320 ul Anhydrous Ethanol** and gently flip the mixture 4 to 6 times to mix well.

* to avoid cross-contamination between samples when the cap is opened, centrifuge at a low speed for several seconds before the cap is opened, so that the solution on the cap can be settled to the bottom of the tube.

5. The solution in step 4 was added to the **Nucleic Acid Purification Column** (the nucleic acid purification column is placed in a 2ml centrifuge tube). Cover with the tube cover.

* be careful not to stick the solution to the edge of the nozzle of the purified column, in case the subsequent washing steps cannot clean the purified column.

6. Centrifuge at 12000 rpm for 30 seconds.

7. Discard the filtrate in the 2 ml centrifuge tube, put the nucleic acid purification column back into the 2 ml centrifuge tube, add **700 ul Buffer WBR** into the nucleic acid purification column, cover the tube, and centrifuge at 12000 rpm for 30 seconds.

* the filtrate does not need to be completely discarded. To avoid contamination of the centrifuge by the filtrate adhering to the nozzle of the centrifuge tube, the 2ml centrifuge tube can be flipped on the paper towel once.
* confirm that anhydrous ethanol has been added to Buffer WBR.

8. Discard the filtrate in the 2 ml centrifuge tube, put the nucleic acid purification column back into the 2ml centrifuge tube, and centrifuge at 14000 rpm for 1 minute.

* if the centrifuge speed cannot reach 14000rpm, centrifuge at the highest speed for 2 minutes.
* please do not omit this step, or the subsequent PCR results may be affected by the presence of ethanol in the purified nucleic acid.

8. Discard the 2ml centrifuge tube, place the nucleic acid purification column in a clean 1.5ml centrifuge tube, add **50 ul** of preheated **Buffer TE** at 56ºC in the center of the membrane of the purified column, cover the tube, let it stand at room temperature for 1 minute, and centrifuge at 12000 rpm for 30 seconds.

* if the centrifuge does not have a leak-proof cover, please change the centrifuge condition to 8000rpm for 1 minute, so as to prevent the tube cover from falling off and damaging the centrifuge.

9. Discard the purified column and store the viral nucleic acid at -20ºC for future use.

Warnings and Precautions

- **This kit is For In-Vitro Diagnostic Use Only**.
  Note the regulatory status in the US, is For Research Use Only. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Handle all specimens as if infectious using safe laboratory procedures.
- Proper personal protective equipment including lab coats, gowns, gloves, eye protection, and a biological safety cabinet are recommended for manipulation of clinical specimens. Refer to CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition https://www.cdc.gov/labs/BMBL.html.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC).
- Laboratories within each country and its territories are required to report all positive results to the appropriate public health authorities.
- PCR-based detection technologies are sensitive to accidental contamination of previous PCR products. False positive results could occur if either the clinical specimen or the real-time reagents become contaminated.
- Perform for assay setup and handling of nucleic acids in separate areas. Workflow in the laboratory should proceed in a unidirectional manner. Use separate and dedicated equipment and supplies in each area.
- Do not substitute or mix reagent from different kit lots or from other manufacturers. Only use aerosol barrier pipette tips and change tips between liquid transfers.
- Good laboratory techniques should be followed to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples. Proper aseptic technique should always be used when working with nucleic acids.
- Wear a clean lab coat and powder-free disposable gloves when setting up assays, and change gloves between samples and whenever contamination is suspected.
- Keep reagent and reaction tubes capped or covered as much as possible.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach, “DNAZap™” or “RNase AWAY®” to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.

- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.
- Dispose of unused kit reagents and human specimens according to local, state, and central regulations.

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