SHIN JIN MEDICS INC.

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Quick Guide

PERFECT SOLUTION FOR DIAGNOSTICS.

SHIN JIN MEDICS INC.

DIAKEY REALcheck Viral DNA/RNA Prep Kit

[For Column Type]

Rev. 2. 2020.08.23

Viral DNA/RNA Prep Kit [For Column Type]

SHIN JIN MEDICS INC.

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SHINJIN MEDICS INC. Quick Guide

DIAKEY REALcheck

Viral DNA/RNA Prep Kit [For Column Type]

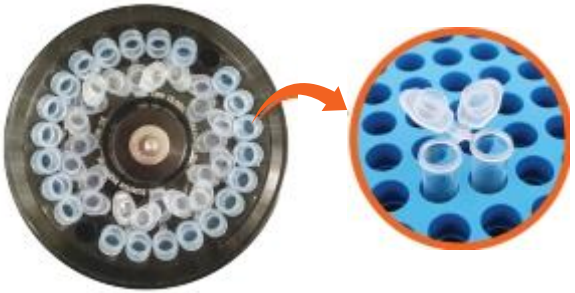
SHIN JIN MEDICS INC.

✓ Components

Components	
VL	30 ㎖
VW-1	60 ㎖
VW-2(100% Ethanol)	Empty bottle (provided)
100% Ethanol	Not provided
RNase free water	10 ㎖
Proteinase K (20 ㎎ / ㎖)	4 EA
Capsule Column	100 preps
Quick Guide	1 EA

✓ Know-How for Preparation

1. How to prevent the cap of the tube from breaking during elution



* Please inset 1.5 ㎖ tubes into centrifuge with their caps crossed to prevent them from breaking.

2. It is recommended to use fresh samples for isolation.

3. Please remove EtOH completely through centrifugation before elution step.

4. Add dried enzyme to D.W(Buffer) and mix well. Please store it at -20° C.

5. Please use the product according to its expiration date.

6. Storage of VL at lower temperature may cause precipitation of salts due to SDS.
   In this case, please use it after thawing in the microwave/dry oven.

7. It is recommended to use RNase free water after pre-heating (10 mins, at 50° C) during elution step for maximal recovery with the Kit.
   (Especially, high efficiency can be achieved in case of large DNA fragment.)

Troubleshooting

Trouble	Check List
Low Yield DNA	**01. Did you use fresh samples?** It is recommended to use fresh virus sample and avoid repeated, multiple freeze-thaw cycles to reduce the risk of viral DNA/RNA degradation. It should be stored in small working aliquots not to dissolve it more than 3 times. **02. Did you use Binding Buffer / VW-2 as 100% EtOH?** Lower percentages of Ethanol or use of other alcohol instead of EtOH may prevent efficient isolation of viral DNA/RNA. Please use 100% Ethanol only. **03. Where did you store the enzyme (Proteinase K)?** Ambient temperatures may harm the enzyme's activity and stability in case of enzyme dissolved in D.W or enzyme with buffer added. It is recommended to be stored at -20° C for long-term storage. **04. How about incubation time after addition of Lysis Buffer?** Short incubation time leads to reduced recovery and it may decrease the yields. Please follow the incubation time specified for this protocol. **05. Did you add RNase free water directly to column membrane?** Make sure that you add RNase free water directly to the membrane during elution step. Please incubate it for 1 min at RT after addition of RNase free water.
Nicked DNA Degraded DNA	**01. Have you thought about nuclease contamination?** Please check all plasticwares such as tips, microcentrifuge tubes and buffers for nuclease contamination before use. Tips and microcentrifuge tubes should be autoclaved to avoid nuclease contamination.
Low Quality DNA	**01. Did you dry Ethanol sufficiently after the washing step?** In case eluted viral DNA/RNA contains Ethanol, it may cause problems for the next experiment. It is recommended to remove Ethanol completely after washing step.

Work Flow

Step 1

Sample **150** ㎕
+ VL(+ 2-ME) **250** ㎕
+ [Option]
Proteinase K (20 ㎎ / ㎖) **20** ㎕

Vortex (30 sec - 1 min)
Incubation (RT, 10 mins)

Step 2

+ 100% Ethanol **350** ㎕
Vortex (30 sec - 1 min)

Add Lysate/Ethanol mix
(Step 2)
in the Capsule column

Centrifugation(13,000 rpm, 1 min)
Flow-through removal
Place the capsule column again

Step 3

+ VW-1 **500** ㎕

Centrifugation(13,000 rpm, 1 min)
Flow-through removal
Place the capsule column again

Step 4

+ VW-2 (100% Ethanol) **500** ㎕

Centrifugation
(13,000 rpm, 1 min)
Flow-through removal

Step 5

Centrifugation again
(13,000 rpm, 1 min)
Removal of residual washing buffer

Place the capsule column
into the new 1.5 ㎖ micro tube

Step 6

+ RNase free water **50** ㎕
Incubation (RT, 1 min)

Centrifugation
(13,000 rpm, 1 min)

DNA/RNA Elution

Viral DNA/RNA Prep Kit [For Column Type]

Preparation

1. DIAKEY REALcheck Viral DNA/RNA Prep Kit is designed to isolate viral DNA/RNA using **150** ㎕ of serum, plasma, cell-culture media and cell free body fluids sample.
(If the sample volume is less than **150** ㎕ , adjust the volume to **150** ㎕ with 1X PBS.)
2. Although viral DNA/RNA is isolated regardless of use of 2-Mercaptoethanol (2-ME) on this kit, it is recommended to add **10** ㎕ of 2-ME (100%, optional) per **1** ㎖ of VL solution (at a ratio of 100:1) prior to use.
Especially, high-quality DNA/RNA can be obtained through rapid inactivation of intracellular RNase and removal of potential PCR inhibitors when using 2-ME. In addition, higher detection sensitivity can be achieved through this treatment during Real-time PCR amplification.
3. Prepare 100% Ethanol (not provided) into VW-2 bottle. Make it fresh.

Protocol

[Cell Lysis]

1 : Add **150** ㎕ Sample to prepared **250** ㎕ VL and perform vortexing for 30 sec – 1 min.
→ Incubate the mixture for 10 mins at RT.

- In case of enveloped virus, please incubate the mixture after addition of 20 ㎕ Proteinase K solution (20 mg/㎖).

[Column Binding & Washing]

2 : Add **350** ㎕ Ethanol (100%) to Lysate (Step 1) and perform vortexing for 30 sec – 1 min.
→ Transfer it into capsule column and perform centrifugation at 13,000 rpm for 1 min
→ Discard the flow-through solution

[Column Washing]

3 : Add **500** ㎕ VW-1 into capsule column and perform centrifugation at 13,000 rpm for 1min.
→ Discard the flow-through solution
4 : Add **500** ㎕ VW-2 (100% Ethanol) into capsule column and perform centrifugation at 13,000 rpm for 1min.
→ Discard the flow-through solution
5 : Perform centrifugation at 13,000 rpm for 1min to remove residual Washing Buffer (Ethanol).
→ Discard collection tube and place the capsule column into the new 1.5 ㎖ micro tube

Protocol

[Viral DNA/RNA Elution]

6 : Add **50** ㎕ RNase free water into the capsule column and incubate it for 1 min at RT
→ Perform centrifugation at 13,000 rpm for 1 min and remove the column
→ Store at -20° C (viral DNA) & store at -70° C (viral RNA)

Guideline for Isolation Precautions

This product should be used by experts of scientific experiments.

Warranty regulations and liability

- Integrity of kit components is guaranteed for up to one and a half years from the date of purchase.
- This warranty is valid only in case the product is used in accordance with the instructions for use.
- This warranty does not extend to any losses or damages due to misuse, negligence or abuse and it will not be replaced.

Safety warnings and safety instructions

- After eye contact: Rinse opened eye for several minutes under running water. The eye should be examined by a doctor if irritation continues after rinse.
- After skin contact: Immediately wash with water and soap. Rinse it thoroughly. The skin should be examined by a doctor if irritation continues after rinse.
- Wear suitable gloves to protect hands from frostbite.

User Instructions

- This product should never be used after the expiration date indicated on the label.
- Repeated freeze-thaw cycles can decrease the activity of Proteinase K. If necessary, store it at -20° C through aliquots into appropriate amounts.
- This product should be operate d in accordance with correct order in the manual and use it immediately after opening.
- Results may vary depending on the condition of isolated DNA/RNA.
- Inaccurate results may be obtained by contaminated samples.

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