AniGen Viral RNA Purification Kit®

■ Principles

AniGen Viral RNA Purification Kit® is designed for rapid isolation of RNA from a variety of sample sources including fresh or frozen plasma, serum, other cell free body fluids and virus-infected cell/tissue. The purified RNA is free of contaminants and impurities, and ideal for PCR and RT-PCR.

AniGen Viral RNA Purification Kit® uses advanced silica-gel membrane technology for rapid and effective purification of RNA without organic extraction or ethanol precipitation. Furthermore, the buffering conditions are finely adjusted to provide optimum binding of the RNA to the column. Procedural directions of **AniGen Viral RNA Purification Kit®** is very simple; users may purify RNA from a variety of target source within 15min.

■ Materials provided

[BOX No. 1]

1. Binding buffer 1*1 .....150ml
2. Binding buffer 2 .....200ml
3. Washing buffer 1.....150ml
4. Washing buffer 2*2 .....50ml
   : Before use, add 200ml of absolute EtOH
5. Rnase-free buffer.....100ml
6. Collection tubes (Polypropylene tube for 2ml).....250EA
7. Instruction for use.....1 sheet

[BOX No. 2]

1. Columns.....250 columns
   : Columns containing silica membrane

*1. Binding buffer 1 is composed high concentration of guanidium salt. The salt of binding buffer is easy to precipitate, when the buffer is stored in low temperature(below 20℃). If the binding buffer become solid, incubate in 80 ℃ for 10 min.

*2. Washing buffer 2 is supplied as concentrates. Add 200ml of ethanol (96~100%) according to bottle label before use.

■ Storage and Stability

Store all components at room temperature(2~30℃)

■ Procedure

1. Transfer 150 (300)㎕ plasma, serum, urine, cell-culture supernatant, cell-free fluid or virus infection tissue or cell in the 1.5㎖ microcentrifuge tube.
   *Note: If sample volume is less than 150㎕, samples should be adjusted to 150㎕ with DEPC treated water.
2. Add 250(500)㎕ of Binding buffer 1
   * Note: If the sample volume is larger than 150㎕, increase the amount of Binding buffer 1 (e.g., a 300㎕ sample will require 500㎕ of Binding buffer 1 and if the Binding buffer 1 become solid, incubate in 80℃ for 10 min.)
3. Mix by vortexing for 15 sec.
4. Incubate at room temperature (15~25℃) for 10 min.
   *Note: If the target virus is composed enveloped structure following the below step for recovery enhancing.
   ①Add 20㎕ of Proteinase K Solution (20㎎/㎖, not provided)
   ②Incubate at 55℃ for 10 min.
5. Add 350(700)㎕ of Binding buffer 2, and completely mix well by gently vortexing.
   * Note: If the sample volume is more than 150㎕, increase the amount ot Binding Buffer 2 (e.g. , a 300㎕ sample will require 700㎕ of Binding buffer 2). This step is conductive efficient passage of cell lysates through a column and to increase binding onto column resins and important for effective deproteinization.
6. Place a spin column in a provided 2㎖ collection tube.
7. Load lysates on the column and centrifuge at 13,000rpm for 1min.
   * Note: The maximum volume of the column reservoirs 800㎕. For sample volumes of more than 800㎕, simply load and spin again. If the solution has not completely passed through the membrane, centrifuge again at higher speed until all of the solution passed through.
8. Discard solution in collection tube and place the column back in the same 2㎖ collection tube.
9. Add 500㎕ of Washing buffer 1 to column and centrifuge for 1min at 13,000rpm.
10. Discard solution in collection tube and place the spin column back in the same 2㎖ collection tube.
11. Add 500㎕ of Washing buffer 2 to the column and centrifuge for 1min at 13,000rpm.
12. Discard solution in collection tube and place the spin column back in the same 2㎖ collection tube. Centrifuge for 1 min at 13,000rpm.
    *Note: It is important to dry the membrane since residual ethanol may interfere with downstream reactions.
13. Place the column in a RNase-free 1.5ml microcentrifuge tube (not provided), and add 30~60㎕ of Rnase-free buffer directly onto the membrane.
14. Incubate at RT for 1min, and then centrifuge for 1 min at 13,000rpm.
15. Use 2~5㎕ of eluted solution for PCR or RT-PCR.

■ Precaution and safety information

All chemicals should be considered as potentially hazardous. When working with chemicals, always were a suitable lab coat and disposable glove. Some buffer contain the chaotrophic salt which may be an irritant and carcinogen, so appropriate safety apparel such as gloves and eye protection should be worn. If a spill of the buffers occurs, clean with a suitable laboratory detergent and water. If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with a suitable laboratory disinfectant. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products.

■ Troubleshooting

Problem	Possible cause	Recommendation
Little or no viral gene in the elute	Low concentration of virus in the sample	Concentrate the sample volume to 300 (150)㎕ using a microconcentrator (Centricom-100 or Microcep 100)
	Inefficient virus lysis in Binding buffer 1	Precipitate, formed in Binding buffer 1 after storage at 15℃ below, was not redissolved by heating before starting the procedure
	Binding buffer 1 prepared incorrectly	Check Binding buffer 1 for precipitate. Dissolve precipitate by incubation at 80℃.
	RNA degraded	Often RNA is degraded by RNases in the starting material. It is recommended to work quickly during sample preparation. If necessary, add RNase inhibitor to the sample.
	Too much starting material (In case of virus infected animal tissues)	Do not overload the sample, overloading significantly reduces purity and yield. After tissues sample homogenization and brief centrifugation, transfer 300 (150)㎕ supernatant to a new tube and add 500(250) ㎕ Binding buffer 1. Do not apply homogenized pellet.
	Binding buffer 2, Washing buffer 1 and 2 used in the wrong order	Ensure that Buffer are used in the correct order in the protocol.
Viral gene does not perform well in subsequent enzymatic reactions	Ethanol carryover	Ensure that after the Washing Buffer 2 wash, the column is spun at maxmum speed for 1 minute to dry the Ani Gen Viral RNA Purification Kit membrane.

DOC. No.: I5090-1
Issude date: Nov. 30.2009

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