ISO 9001/14001 Certified Company

コスモ・バイオ株式会社

IQeasy Plus Viral DNA/RNA Extraction Kit

Instruction Manual

For extraction of Viral DNA/RNA.

For research use only. Not for use in diagnostic procedures.

Cat. No. 17153 | 50 Columns

Ver 1.0

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9. NOTICE

- For research purpose only. Not for use in diagnostic procedures for clinical purposes. *For in Vitro Use Only*.
- Do not use any reagent after the expiration date.
- Do not use together with reagents of other products.
- Follow the instructions.

10. TROUBLE SHOOTING

Problem	Possible Cause	Recommendation
Little or no viral gene in the eluate	Low concentration of virus in the sample	- Concentrate the sample volume to 300 (150) µl using a microconcentrator (Centricom-100 or Microcep 100).
	Inefficient virus lysis in Lysis Buffer	- Precipitate, formed in Lysis Buffer after storage at 15℃ below, was not redissolved by heating before starting the procedure
	Lysis Buffer prepared incorrectly	- Check Lysis Buffer 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)µl super-natant to a new tube and add 500 (250) µl Lysis buffer. Do not apply homogenized pellet.
	Buffer binding, washing A and washing B 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 B wash, the column is spun at maximum speed for 1minute to dry the membrane of Spin Column

8. EXPERIMENTAL DATA

■ **Extraction efficiency comparison with Viral Gene-spin™ Viral DNA/RNA Extraction Kit and Competitor's**

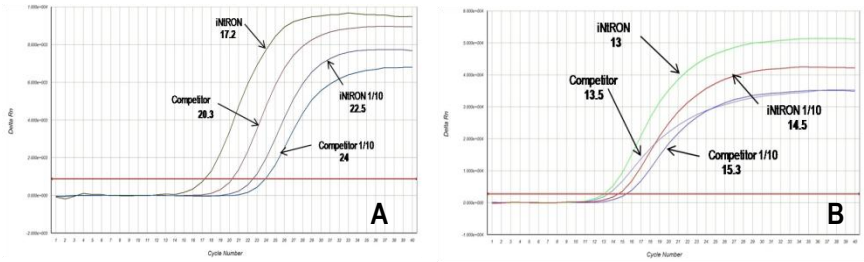

Figure 1. Comparison of viral genomic extraction efficiency of IQeasy Plus Viral DNA/RNA Extraction Kit with competitor's

Influenza virus samples were used 1X or 1/10X diluted with PBS Buffer, than viral gene from the samples were extraction with IQeasy Plus Viral DNA/RNA Extraction Kit or competitor's. After extraction, each of 2 µl of extracted viral genes were used as template of Real-time RT-PCR analysis. The threshold of cyle (Ct) value from IQeasy Plus Viral DNA/RNA Extraction Kit shows lower than competitor's.

A, Amplification curve of influenza A gene detection; **B,** amplification curve of Rnase P gene detection as internal control.

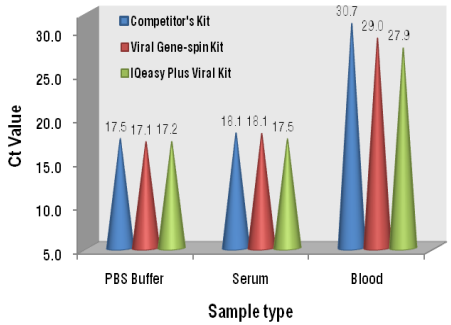

Figure 2. Comparison of threshold of cycle (Ct) value of IQeasy Plus Viral DNA/RNA Extraction Kit with Viral gene-spin Viral DNA/RNA Extraction Kit and competitor's

The three kinds of virus infected (or mixed) samples were used as start specimens for viral gene extraction. In results, the Ct value of IQeasy Plus Viral DNA/RNA Extraction Kit shows lower than other kits.

1. DESCRIPTION

IQeasy Plus Viral DNA/RNA Extraction Kit is designed for rapid and sensitive isolation of DNA or 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 DNA/RNA is free of contaminants and impurities, and ideal for PCR and RT-PCR.

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

2. STORAGE

Proteinase K (-20 ℃), Binding Carrier (Preserve at 4 ℃ after receiving) Other components at RT.

3. KIT CONTENTS

Label	Description	Contents
Lysis Buffer[1]		30 ml
Binding Buffer		40 ml
Washing Buffer A		60 ml
Washing Buffer B[2] (concentrate)	Add 40ml of EtOH before use	10 ml
Elution Buffer	Elution Buffer	20 ml
Spin Columns (Orange color column)	Inserted into a collection tubes. (2.0ml tubes)	50 columns
Proteinase K	20 mg/ml of concentration	0.5 ml
Binding Carrier	Co-binding carrier for a few amount of nucleic acid binding	0.4 ml

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

[2] Washing Buffer B is supplied as concentrates. Add 40 ml of ethanol (96~100%) according to the bottle label before use.

4. PRECAUTIONS AND SAFETY INFORMATION

All chemicals should be considered as potentially hazardous. When working with chemicals, always wear 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.

5. PRODUCT WARRANTY AND SATISFACTION GUARANTEE

All products undergo extensive quality control test and are warranted to perform as described when used correctly. Immediately any problems should be reported. Satisfaction guarantee is conditional upon the customer providing full details of the problem to iNtRON within 60 days, and returning the product to iNtRON for examination.

6. ADDITIONAL REQUIRED MATERIALS

- Disposable gloves
- Pipettes
- Vortex mixer
- Thermal cycler
- Sterile pipette tip
- Centrifuge for microcentrifuge tubes
- Electrophoresis kit / UV transilluminator

7. PROCEDURE

Please read through the entire procedure before starting.

1) Transfer 150 (300) µl plasma, serum, urine, cell-culture supernatant, cell-free fluid or virus infection tissue or cell in the 1.5ml microcentrifuge tube.
   Note : If sample volume is less than 150 µl, sample should be adjusted to 150 µl with DEPC treated water.

2) Add 250 (500) µl of Lysis Buffer, 10 µl of Proteinase K, 7.5 µl of Binding Carrier.
   Note: If the sample volume is larger than 150 µl, increase the amount of Lysis buffer (e.g., a 300 µl sample will require 500 µl of Lysis buffer) and if the Lysis buffer become solid, incubate in 80 °C for 10min.

3) Mix by vortexing for 15sec.

4) Incubate at 56 °C for 10 min.

5) Add 350 (700) µl of Binding Buffer, and completely mix well by gently vortexing.
   Note : If the sample volume is more than 150 µl, increase the amount of Binding buffer (e.g., a 300 µl sample will require 700 µl of Binding buffer). This step is conducive 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 ml collection tube.

7) Load lysates on the column and centrifuge at 13,000 rpm for 1 min.
   Note : The maximum volume of the column reservoirs 800 µl. For sample volumes of more then 800 µl, 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 ml collection tube.

9) Add 500 µl of Washing Buffer A to column and centrifuge for 1 min at 13,000 rpm.

10) Discard solution in collection tube and place the spin column back in the same 2 ml collection tube.

11) Add 500 µl of Washing Buffer B to the column and centrifuge for 1 min at 13,000 rpm.

12) Discard solution in collection tube and and place the spin column back in the same 2ml collection tube. Centrifuge for 1 min at 13,000 rpm.
    Note : It is important to dry the membrane since residual ethanol may interfere with downstream reactions.

13) Place the column in a RNase-free 1.5 ml microcentrifuge tube (not provided), and add 30-60 µl of Elution Buffer directly onto the membrane.

14) Incubate at RT for 1 min, and then centrifuge for 1 min at 13,000 rpm.

15) Use 2-5 µl of eluted solution for PCR or RT-PCR.