

E.Z.N.A.® Viral RNA Kit

– Instruction Manual –

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Introduction

E.Z.N.A.® Viral RNA Kit is designed for isolation of Viral RNA from cell free fluids such as plasma, serum, urine, and cell culture supernatants.

The procedure completely removes contaminants and enzyme inhibitors, making viral RNA isolation fast, convenient, and reliable. This kit has been tested for isolating viral nucleic acids from hepatitis A, C, and HIV. The kit is also suitable for isolation of total RNA from cultured cells, tissues, and bacteria.

RNA purified using the E.Z.N.A.® Viral RNA method is ready for applications such as RT-PCR*.

Theory

The E.Z.N.A.® Viral RNA Kits use reversible binding properties of HiBind® matrix, a new silica-based, time saving spin technology material. Combined with the speed of mini-column spin technology or vacuum manifold, multiple samples can be processed at the same time. The sample is lysed first under highly denaturing buffer conditions so that RNases will be inactivated, and the intact viral RNA is protected from degrading. After adjusting the buffer condition, the samples are loaded to the HiBind® RNA column. With a brief centrifugation or vacuum, the samples pass through the column and the viral RNA binds to the HiBind® matrix. After two washing steps, purified viral RNA will be eluted with RNase-free water.

Note

E.Z.N.A.® Viral RNA Kits are not designed to separate viral RNA from cellular RNA and DNA. It will purify both in parallel if they present in the sample. Cell free body fluids are recommended.

* PCR is covered by patents owned by F. Hoffmann-La Roche Ltd.

Kit Components

E.Z.N.A.® Viral RNA Kit	5 Purifications	50 Purifications	200 Purifications
Product Number	12-6874-00	12-6874-01	12-6874-02
Components			
HiBind® Columns	5	50	200
2 ml Collection Tubes	15	150	600
QVL Buffer	5 ml	30 ml	120 ml
RNA Wash buffer I	5 ml	40 ml	200 ml
RNA Wash buffer II	5 ml	12 ml	50 ml
Carrier RNA	50 µg	400 µg	1600 µg
DEPC-ddH ₂ O	1.5 ml	10 ml	30 ml
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Storage and Stability

E.Z.N.A.® Viral RNA Kit components should be stored at room temperature (22 °C – 25 °C). All E.Z.N.A.® Viral RNA Kit components are stable for at least 12 months from the date of purchase when stored at 22-25 °C. During shipment crystals may form in the QVL Buffer. Warm up to 37 °C to dissolve.

Before Starting

Briefly examine this booklet and become familiar with each step. Prepare all components and have the necessary materials ready before starting.

- ! Carrier RNA dissolved in QVL Buffer must be stored at 2-8°C, and it should be stable for up to 6 months. QVL/carrier RNA solution is only stable for 10 days at room temperature. DO NOT frequently warm up QVL/Carrier RNA solution. It is recommended to make aliquots of this buffer according to average usage per week.
- ! Whenever working with RNA, always wear latex gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents. Work carefully but as quickly as possible during the procedure.
- ! Under cool ambient conditions, crystals may form in the QVL Buffer. This is normal and the bottle should be warmed (37 °C) to dissolve the salt before use.
- ! Sample volume: HiBind® RNA spin column can bind any RNA greater than 200nt. Yield will depend on the sample sources and conditions. The protocol is optimized for use with 150 :l samples. Smaller samples should be adjusted to 150 µl with PBS or DEPC water; lower titer samples should be concentrated to 150 µl before processing. For samples larger than 150 µl, the amount of QVL Lysis buffer and other reagents added to the sample before loading must be increased proportionally.
- ! RNA Wash Buffer II is concentrated and has to be diluted with absolute ethanol as follows:

Kit 12-6874-00	Add 20 ml 100% EtOH to 5 ml Wash Buffer II
Kit 12-6874-01	Add 96 ml 100% EtOH to 24 ml Wash Buffer II
Kit 12-6874-02	Add 200 ml 100% EtOH to 50 ml Wash Buffer II

Store diluted DNA Wash Buffer at room temperature.

- ! Adding Carrier RNA to Buffer QVL Lysis buffer: add 1ml QVL Buffer to the tube of lyophilized Carrier RNA. Completely dissolve Carrier RNA and transfer the mixture to the Buffer QVL buffer bottle. Mix thoroughly by shake few times.

E.Z.N.A.® Viral RNA Isolation Protocol

Materials required, but not supplied:

! 96-100% ethanol

! Sterile RNase-free pipette tips and microcentrifuge tubes

! Table top microcentrifuge at room temperature.

! Disposable latex gloves

***Note:** Equilibrate samples and QVL buffer to room temperature before beginning. All steps must be carried out at room temperature. Work quickly, but carefully.*

1. Lysis

Add 500 µl QVL Lysis buffer into a 1.5 ml microcentrifuge tube.

Pipet 150 µl plasma, cell free body fluid, cell culture or urine into the microcentrifuge containing QVL/Carrier RNA. Mix thoroughly by vortexing for 30 seconds.

Incubate at room temperature for 5-10 minutes.

Spin briefly to collect any liquid from lid. Add 350 µl of absolute ethanol (96-100%) to the sample, mix thoroughly by vortexing for 30 seconds. Centrifuge briefly to collect any liquid droplets from lid.

2. Load and bind

Apply the 750 µl of sample (including any precipitate) to a HiBind® RNA column assembled in a 2 ml collection tube (supplied).

The maximum capacity of the HiBind™ RNA spin cartridge is 800 µl. During the procedure, work carefully but quickly. (Larger volumes can be loaded successively.) Centrifuge at 10,000 x g for 15 seconds. Discard flow-through. Repeat this step until all the lysate has been loaded on to the spin column.

3. Wash I

Wash column with Wash Buffer I by pipetting 750 µl directly into the spin column. Centrifuge as above and discard the 2 ml collection tube.

4. Wash II

Place column in a clean 2ml collection tube (supplied), and add 500 µl Wash Buffer II diluted with ethanol. Centrifuge and discard flow-through. Reuse the collection tube in step

6. Dry (Important, do not skip this step!)

Place the HiBind® spin column containing your RNA in the collection tube used in step 5 and centrifuge for 1 min at 10,000 x g to dry the column matrix. This step is essential to remove ethanol from the column.

7. Elution

Transfer the column to a clean 1.5 ml microfuge tube (not supplied with kit) and elute the RNA with 50-100 µl of DEPC-treated water (supplied with kit). Make sure to add water directly onto column matrix. Centrifuge 1 min at maximum speed.

DNA Contamination

No RNA extraction procedure can completely remove genomic DNA. For sensitive work (such as RT-PCR* or differential display) we suggest that you treat the eluted RNA with RNase-free DNase. Also for RT-PCR, use intron-spanning primers that allow easy identification of DNA-contamination. Using RNA as a template in a control PCR* reaction will also allow the detection of DNA contamination.

* PCR is covered by patents owned by F. Hoffmann-La Roche Ltd.

Quantitation and Storage of RNA

Determine the absorption of an appropriate dilution (10- to 50-fold) of the sample at 260 nm and then at 280 nm.

DEPC-water is slightly acidic and can dramatically lower absorption values. We suggest that you dilute the sample in a buffered solution (TE) for spectrophotometer analysis.

One A_{260} -unit is about 40 μg RNA/ml. The RNA concentration is calculated as follows:

$$\text{RNA conc. } (\mu\text{g / ml}) = \text{Absorption}_{260} \times 40 \times \text{Dilution Factor}$$

The ratio of $A_{260/280}$ is an indication of nucleic acid purity. Values higher than 1.8 indicates > 90% nucleic acid.

Phenol has an absorption maximum at 275 nm and can interfere with absorption readings of DNA or RNA. However, the E.Z.N.A.[®] Plant RNA Kit eliminates the use of phenol and avoids this problem.

Store RNA samples at $-70\text{ }^{\circ}\text{C}$ in sterile DEPC- dH_2O . Under such conditions RNA prepared with the E.Z.N.A.[®] system is stable for at least one year.

RNA Quality

It is highly recommended to determine the RNA quality prior to further applications. Denaturing agarose gel electrophoresis and ethidium bromide staining can best assess the quality of RNA. Two sharp bands should appear on the gel. These are the 28S and 18S ribosomal RNA bands. If these bands smear towards lower molecular weight RNA, then the RNA has undergone major degradation during preparation, handling, or storage.

Although RNA molecules less than 200 bases in length do not efficiently bind the HiBind[®] matrix, a third RNA band, the tRNA band, may be visible when a large number of cells are used.

Ordering Information

For RNA isolation from cells, tissues and blood:

E.Z.N.A.® Plant RNA Kit	12-6627-00	5 Preparations
	12-6627-01	50 Preparations
	12-6627-02	200 Preparations
E.Z.N.A.® Bacterial RNA Kit	12-6850-00	5 Preparations
	12-6850-01	50 Preparations
	12-6850-02	200 Preparations
E.Z.N.A.® Viral RNA Kit	12-6874-00	5 Preparations
	12-6874-01	50 Preparations
	12-6874-02	200 Preparations
E.Z.N.A.® Total RNA Kit (Safety-Line)	12-6834-00	5 Preparations
	12-6834-01	50 Preparations
	12-6834-02	200 Preparations
E.Z.N.A.® Total RNA Kit (Classic-Line)	12-6634-00	5 Preparations
	12-6634-01	50 Preparations
	12-6634-02	200 Preparations
E.Z.N.A.® Blood RNA Kit (Safety-Line)	12-6814-00	5 Preparations
	12-6814-01	50 Preparations
	12-6814-02	200 Preparations
E.Z.N.A.® Blood RNA Kit (Classic-Line)	12-6614-00	5 Preparations
	12-6614-01	50 Preparations
	12-6614-02	200 Preparations

Troubleshooting Tips

Problem	Likely cause	Suggestion
Little or no RNA eluted	RNA remains on the column	<ul style="list-style-type: none"> Repeat elution. Pre-heat DEPC-water to 70° C prior to elution. Incubate column for 5 min with water prior to centrifugation.
	Carrier RNA not added to QVL Buffer or degraded	<ul style="list-style-type: none"> Dissolve the carrier RNA with QVL Buffer and repeat the purification with few sample. Avoid warm the QVL/Carrier RNA frequently.
	Column is overloaded	<ul style="list-style-type: none"> Reduce quantity of starting material.
Clogged column	Incomplete lysis	<ul style="list-style-type: none"> Mix thoroughly after addition of QVL Lysis Buffer Increase centrifugation time. Reduce amount of starting material
Degraded RNA	Source	<ul style="list-style-type: none"> Do not freeze and thaw samples more than once. Follow protocol closely, and work quickly. Low concentration of virus in the sample.
	RNase contamination	<ul style="list-style-type: none"> Ensure not to introduce RNase during the procedure. Check buffers for RNase contamination.
Problem in downstream applications	Salt carry-over during elution	<ul style="list-style-type: none"> Ensure Wash Buffer II has been diluted with 100% ethanol as indicated on bottle. Diluted Wash Buffer II must be stored at room temperature. Repeat wash with Wash Buffer II.
DNA contamination		<ul style="list-style-type: none"> Digest with RNase-free DNase and incubate at 37°C for 5 min.