ZYMO RESEARCH

RNA Purification Made Simple™

Quick-RNA™ Viral Kit

Viral RNA from any biological sample

Highlights

- Quick, spin-column purification of viral RNA from plasma, serum, urine, cell culture media, blood, saliva, cellular suspensions, swab, fecal and biopsy samples
- High-quality RNA is ready for Next-Gen sequencing, RT-qPCR, hybridization, etc.
- DNA/RNA Shield is included for sample collection, inactivation, storage and preservation.

Catalog Numbers:
R1034, R1035

Scan with your smart-phone camera to view the online protocol/video.

Table of Contents

Product Contents

Quick-RNA™ Viral Kit	R1034 (50 prep)	R1035 (200 prep)
DNA/RNA Shield™ (2X concentrate)	25 ml	125 ml
Viral RNA Buffer[1]	50 ml	100 ml (x2)
Viral Wash Buffer[2] (concentrate)	6 ml (x2)	48 ml
DNase/RNase-Free Water	4 ml	10 ml
Zymo-Spin™ IC Columns	50	200
Collection Tubes	100	400
Instruction Manual	1 pc	1 pc

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature (15-30°C)
Before use:
1 Add beta-mercaptoethanol (β-Me; user provided) to 0.5% (v/v) *i.e.,* add 250 µl or 500 µl β-Me per 50 ml or 100 ml **Viral RNA Buffer.**
2 Add 24 ml of 100% ethanol (26 ml of 95% ethanol) to the 6 ml **Viral Wash Buffer** concentrate (R1034) or 192 ml of 100% ethanol (204 ml of 95% ethanol) to the 48 ml **Viral Wash Buffer** concentrate (R1035).

Specifications

- **Sample Sources –** ≤ 400 µl plasma, serum, saliva, swab, urine, cell culture media, blood, cellular suspension, fecal sample or ≤ 5 mg biopsy sample.

  For samples in UTM®/VTM®, PBS or saline, see Sample Preparation, page 5.

- **Purity –** RNA is ready for Next-Gen Sequencing, RT-qPCR, etc.
- **Binding Capacity –** 10 µg total RNA (**Zymo-Spin™ IC Columns**).
- **Elution Volume –** ≥ 6 µl **DNase/RNase-Free Water**.
- **Equipment Needed** (user provided) **–** Beta-mercaptoethanol (b-Me), Ethanol (95-100%), Microcentrifuge.
- **Materials** (available separately) **–**

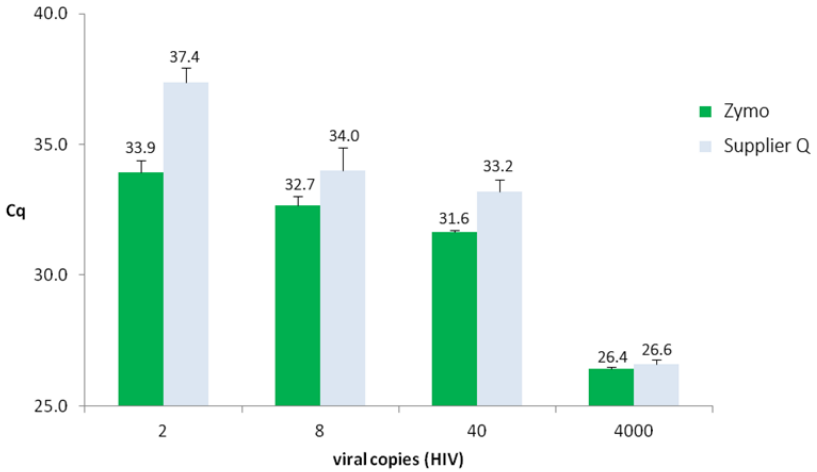
  DNase I Set (E1010; 50 rxns.; 250 U DNase I (lyophilized) supplied w/ DNA Digestion Buffer, 4 ml)
  RNA Prep Buffer (R1060-2-50; 50 ml)
  RNA Wash Buffer (concentrate) (R1003-3-6, 6 ml)
  Proteinase K Set (D3001-2-20; 20 mg Proteinase K (lyophilized) supplied w/ Storage Buffer).

Product Description

The ***Quick*-RNA™ Viral Kit** is a quick, purification of viral RNA from plasma, serum, urine, cell culture media, blood, saliva, cellular suspensions, biopsies, swab and fecal samples stored in **DNA/RNA Shield**™ (for sample collection, nucleic acid preservation and inactivation of pathogens).

The kit also features a buffer system that facilitates complete viral particle lysis for efficient nucleic acid isolation. Small (> 50 nt) and large (> 200 kb) DNA and RNA are bound to the column, washed and eluted.

The isolated high-quality, total RNA is ready for all downstream applications such as Next-Gen sequencing, hybridization-based and RT-qPCR detection.



The ***Quick*-RNA™ Viral Kit** from Zymo Research ensures high sensitivity viral detection compared to that of Supplier Q. Viral RNA was isolated from plasma samples. Data shows the mean (+/- SD) of triplicate RT-qPCR measurements.

Protocol

The protocol consists of: (I) Buffer Preparation, (II) Sample Preparation and (III) RNA Purification.

(I) Buffer Preparation

- ✓ Add beta-mercaptoethanol (user provided) to 0.5% (v/v) i.e., add 250 µl or 500 µl β-Me per 50 ml or 100 ml **Viral RNA Buffer.**
- ✓ Add 24 ml of 100% ethanol (26 ml of 95% ethanol) to the 6 ml **Viral Wash Buffer** concentrate (R1034) or 192 ml of 100% ethanol (204 ml of 95% ethanol) to the 48 ml **Viral Wash Buffer** concentrate (R1035).

(II) Sample Preparation

- ✓ Perform all steps at room temperature (15-30°C).
- ✓ Depending on sample type, up to 400 µl can be processed per prep (see below).

Samples in DNA/RNA Shield™[1,2] collection devices (swabs, saliva, etc.)
Transfer up to 400 µl and proceed directly with purification, page 6.

Swabs (UTM®/VTM®, PBS, saline, etc.)
Transfer up to 400 µl and proceed directly with purification, page 6.
Optional - To inactivate, store and preserve samples at room temperature prior to further processing, add **DNA/RNA Shield™**. See **Liquids**, below.

Liquids (plasma[2], serum[2], CSF, blood, saliva, urine, cell suspension, cell culture media)
Add 200 µl of DNA/RNA Shield™ (2X concentrate) to 200 µl liquid sample (1:1) and mix well. Transfer up to 400 µl of the mixture and proceed with purification, page 6.

Tissue[2] (LCM, needle biopsy)
Add 400 µl **DNA/RNA Shield™** (1X) to a tissue sample (up to 5 mg) and mix well. Proceed with purification, page 6.

Optional - **Proteinase K treatment**[3] (protein-rich samples e.g., plasma, serum, saliva, sputum, tissue, can be treated). Materials sold separately
Add 1% **Proteinase K** (v/v) at 20 mg/ml directly to a liquid sample. Mix well and incubate at room temperature for 15 minutes. Note: Up to 5% Proteinase K can be added (e.g., tissue). For example: Add 4-20 µl Proteinase K to each 400 µl sample.

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1 At this point, samples in DNA/RNA Shield™ can be stored at ambient temperature (4-30°C) for a month, 7 days at 37°C, or long-term (> 1 year) -20°C or below.
2 To remove particulate debris or cryoprecipitates (if any), centrifuge and transfer up to 400 µl of the cleared supernatant into a nuclease-free plate/tube (not provided).
3 Prior to use, reconstitute the lyophilized Proteinase K (D3001-2-20) and add 1,040 µl Storage Buffer. Mix well and store frozen aliquots.

(III) RNA Purification

- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g.
- ✓ The sample input can be scaled up or down, proportionally.

1. Add 800 µl **Viral RNA Buffer** to each 400 µl sample[1] (2:1) and mix well.
2. Transfer the mixture into a **Zymo-Spin™ IC Column**[2] in a **Collection Tube** and centrifuge for 2 minutes. Transfer the column into a **new** collection tube.

   Optional: At this point, DNase I treatment can be performed (see Appendices, page 7).
3. Add 500 µl **Viral Wash Buffer** to the column, centrifuge for 30 seconds and discard the flow-through. Repeat this step.
4. Add 500 µl ethanol (95-100%) to the column and centrifuge for 1 minute to ensure complete removal of the wash buffer. Carefully, transfer the column into a nuclease-free tube (not provided).
5. To elute RNA, add 15 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge for 30 seconds.

   Alternatively, for highly concentrated RNA use ≥ 6 µl elution.

   The eluted RNA[3] can be used immediately or stored frozen.

1 Up to 400 µl sample (including the volume of DNA/RNA Shield, if added) can be processed per prep.
2 To process > 700 µl, the column can be reloaded.
3 It is recommended to titrate the RNA eluate for downstream applications (i.e., RT/qPCR, etc.).

Appendices

DNase I Treatment

✓ For DNA-free RNA, DNase I treatment can be performed using DNase I Set (E1010; 50 reactions), RNA Prep Buffer (R1060-2-50) and RNA Wash Buffer (concentrate) (R1003-3-6); materials sold separately.

For each sample to be treated, prepare **DNase I Reaction Mix** in an RNase-free tube (not provided) and mix by gentle inversion:

DNase I Reaction Mix	
DNA Digestion Buffer	35 µl
DNase I (reconstituted; 1 U/ul)[1,2]	5 µl

1. Following RNA binding (page 6, step 2), add 400 µl **RNA Wash Buffer**[3] to the column, centrifuge and discard the flow-through.
2. Add 40 µl **DNase I Reaction Mix** directly to the matrix of the column.
3. Incubate at room temperature for (15-30°C) for 15 minutes.
4. Add 500 µl **RNA Prep Buffer** to the column, centrifuge and discard the flow-through.
5. Proceed with RNA Purification (page 6, step 3).

1 Prior to use, reconstitute lyophilized 250 U **DNase I** (E1009-A) to 1U/µl (final concentration) with 275 µl nuclease-free water (not provided), mix by gentle inversion and store frozen aliquots.
2 Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A260 units/ml of reaction mixture at 25°C.
3 Before use, add 24 ml of 100% ethanol (26 ml of 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate.

Ordering Information

Product Description	Catalog No.	Size
***Quick*-RNA™ Viral Kit**	R1034 R1035	50 preps. 200 preps.

Individual Kit Components	Catalog No.	Amount
DNA/RNA Shield™ (2X concentrate)	R1200-25 R1200-125	25 ml 125 ml
Viral RNA Buffer	R1034-1-50 R1034-1-100	50 ml 100 ml
Viral Wash Buffer (concentrate)	R1034-2-24 R1034-2-48	24 ml 48 ml
Zymo-Spin™ IC Columns	C1004-50 C1004-250	50 250
Collection Tubes	C1001-50 C1001-500	50 500
DNase/RNase-Free Water	W1001-30 W1001-100	30 ml 100 ml
DNA/RNA Shield™ Fecal Collection Tube	R1101	10
DNA/RNA Shield™ Collection Tube **DNA/RNA Shield™ Lysis Tube** (microbe) **DNA/RNA Shield™ Lysis Tube** (microbe) w/ swab **DNA/RNA Shield™ Lysis Tube** (tissue)	R1102 R1103 R1104 R1105	50 50 50 50
DNA/RNA Shield™ Collection Tube w/ Swab (1 ml fill)	R1106 R1107	10 50
DNA/RNA Shield™ Collection Tube w/ Swab (2 ml fill)	R1108 R1109	10 50
DNA/RNA Shield™ Saliva Collection Kit (2 ml fill)	R1210	1
DNase I Set (250 U DNase I (lyophilized) supplied with DNA Digestion Buffer, 4 ml)	E1010	1
RNA Prep Buffer	R1060-2-25 R1060-2-50	25 ml 50 ml
RNA Wash Buffer	R1003-3-6 R1003-3-24	6 ml 24 ml
Proteinase K Set supplied w/ Storage Buffer	D3001-2-5 D3001-2-20	5 mg 20 mg

Complete Your Workflow

✓ For sample collection, inactivation of pathogens, storage and preservation of nucleic acids, use DNA/RNA Shield™ collection devices:

DNA/RNA Shield™ Collection Devices	
DNA/RNA Shield™ Collection Tube w/ Swab (1 ml fill or 2 ml fill) #R1107, R1109	For swab samples of nasal, throat, etc.
DNA/RNA Shield™ Saliva Collection Kit (2 ml fill) #R1210	For saliva, sputum, etc.
DNA/RNA Shield™ Collection Tube DNA/RNA Shield™ Lysis Tube (microbe) DNA/RNA Shield™ Lysis Tube (microbe) w/ swab DNA/RNA Shield™ Lysis Tube (tissue) #R1102-R1105	For microbes, tissue, etc. (2 ml lysis tubes used for bead beating homogenization)

✓ For RNA clean-up (purification) from the aqueous phase (e.g., TRIzol, TRI Reagent or similar) or from any enzymatic reaction (e.g., DNase I treated RNA):

RNA Clean & Concentrator	
Microprep #R1013, R1015	DNase I Set included (#R1013)
MagBeads #R1081, R1082	(#R1082)

Troubleshooting Guide

Problem	Possible Causes and Suggested Solutions
RNA degradation	To prevent RNA degradation: Immediately collect and lyse fresh sample into a stabilization reagent (i.e., DNA/RNA Shield™) to ensure nucleic acid stability. Homogenized samples in DNA/RNA Shield™ can be stored frozen for later processing.
Low nucleic acid content and/or low sensitivity in downstream application	Incomplete deproteinization due to high-protein content in the sample (blood, plasma/serum, tissue etc.): - Increase the volume of DNA/RNA Shield™ to the sample. - Perform Proteinase K treatment (see Sample Preparation, page 4). Increase eluate input: -Titrate the DNA/RNA eluate for downstream applications (i.e., RT/qPCR).
DNA contamination	To remove DNA: - Perform DNase I treatment during the purification (page 6) or perform DNase I treatment post-purification (#R1013), then clean-up the treated sample.

For technical assistance, please contact 1-888-882-9682 or email tech@zymoresearch.com

Notes

Notes

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