ZYMO RESEARCH

RNA Purification Made Simple™

Quick-RNA™ Viral 96 Kit

Viral RNA from any biological sample

Highlights

- Spin-plate (96-well) 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:
R1040-E, R1041-E

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

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Product Contents

Quick-RNA™ Viral 96 Kit	R1040-E (2 x 96 prep)	R1041-E (4 x 96 prep)
DNA/RNA Shield™ (2X concentrate)	125 ml	125 ml (x2)
Viral RNA Buffer[1]	100 ml (x2)	100 ml (x4)
Viral Wash Buffer[2] (concentrate)	48 ml	48 ml (x2)
DNase/RNase-Free Water	4 ml	4 ml (x2)
Zymo-Spin™ I-96 Plate	2	4
Collection Plate	2	4
Elution Plate	2	4
96-Well Plate Cover Foil	2	4
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 500 µl β-Me per 100 ml **Viral RNA Buffer.**
2 Add 192 ml of 100% ethanol (204 ml of 95% ethanol) to the 48 ml **Viral Wash Buffer** concentrate.

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™ I-96 Plate**).
- **Elution Volume –** ≥ 10 µl **DNase/RNase-Free Water**.
- **Equipment Needed** (user provided) **–** Beta-mercaptoethanol (b-Me), Ethanol (95-100%), Centrifuge with 96-well plate carrier.
- **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) (R1003-3-12, 12 ml)
  Proteinase K Set (D3001-2-20; 20 mg Proteinase K (lyophilized) supplied w/ Storage Buffer).

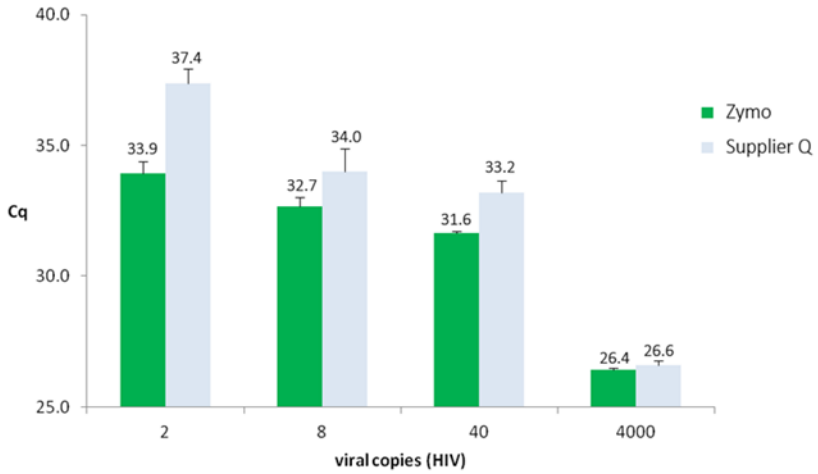
- **Storage Temperature and Stability**
  - ✓ Store all components (i.e., buffers/reagents, columns) at room temperature (15-30°C).
  - ✓ Expiration dates for each of the unopened components are indicated on the individual component labels. These storage conditions apply to both opened and unopened components.
  - ✓ Eluted DNA/RNA can be used immediately or stored frozen (-20/-80°C).

Product Description

The ***Quick*-RNA™ Viral 96 Kit** is a spin-plate (96-well) 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 each well of the plate, 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.

General Laboratory Warnings/Precautions

This assay is for *in vitro* diagnostic use. Nucleic extraction kits are designed for procedures of molecular diagnostic and can only be handled by personal trained in molecular biology methods.

- ✓ This product is intended for professional use only. DO NOT use if the product is visibly damaged.
- ✓ Wear gloves when handling specimens or reagents.
- ✓ Do not pipette by mouth.
- ✓ Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- ✓ Clean and disinfect spills of specimens by including the use of soap and water (i.e., 20% aqueous solution of Sodium Dodecyl Sulfate disinfectant (SDS)).
- ✓ Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the relevant regulatory authority in which the user and/or the patient is established.
- ✓ Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and European regulations.

  Important information regarding the safe handling, transport, and disposal of this product is contained in the Safety Data Sheet. Safety Data Sheets are available from Zymo Research Corp. Inquire directly.

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 500 µl β-Me per 100 ml **Viral RNA Buffer**.
- ✓ Add 192 ml of 100% ethanol (204 ml of 95% ethanol) to the 48 ml **Viral Wash Buffer** concentrate.

(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 7.

Swabs (UTM®/VTM®, PBS, saline, etc.)
Transfer up to 400 µl and proceed directly with purification, page 7.
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 7.

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 7.

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 35°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 (15-30°C) and centrifugation at 3,000-5,000 x g for 5 minutes.
- ✓ The sample input can be scaled up or down, proportionally.
- ✓ Do not use the **96-Well Cover Foil** on the spin-plate during RNA Purification. If necessary, use an Air Permeable Sealing Cover (#C2011-8); sold separately.

1. Add 800 µl **Viral RNA Buffer** to each 400 µl sample[1] (2:1) and mix well.
2. Transfer the mixture into each well of the **Zymo-Spin™ I-96 Plate**[2] mounted on a **Collection Plate** and centrifuge. Discard the flow-through from the collection plate.

   Optional: At this point, DNase I treatment can be performed (see Appendices, page 8).
3. Add 500 µl **Viral Wash Buffer** to each well, centrifuge and discard the flow-through. Repeat this step.
4. Add 500 µl ethanol (95-100%) to each well and centrifuge. Then mount the spin-plate onto an **Elution Plate**.
5. To elute RNA, add 15 µl **DNase/RNase-Free Water** directly to the matrix of each well and centrifuge.

   Alternatively, for highly concentrated RNA use ≥ 10 µl elution.

   The eluted RNA[3] can be used immediately or stored frozen.
   Use the **96-Well Cover Foil** to prevent the eluate from evaporation.

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 plate can be reloaded.
3 It is recommended to titrate the DNA/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) (R1003-3-12); 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 7, step 2), add 400 µl **RNA Wash Buffer**[3] to each well, centrifuge the plate and discard the flow-through.
2. Add 40 µl **DNase I Reaction Mix** directly to the matrix of each well.
3. Incubate at room temperature for (15-30°C) for 15 minutes.
4. Add 500 µl **RNA Prep Buffer** to each well, centrifuge the plate and discard the flow-through.
5. Proceed with RNA Purification (page 7, 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 48 ml of 100% ethanol (52 ml of 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate.

Performance

Repeatability

Repeatability was performed with 1 specific manufactured lot of reagents in the *Quick*-RNA™ Viral 96 Kit (i.e., batch 0). Batch 0 was tested 3 times, each in triplicate preps, for the extraction of a standard viral RNA control diluted in plasma and quantified by RT-qPCR according to quality control, standard operating procedure (R1040-E/R1041-E). For intra-assay reproducibility, global mean value and individual test values are consistent. Standard variation is consistent over 3 runs of the same batch (table 1).

Intermediate Fidelity

Intermediate fidelity was performed with 4 specific manufactured lots of reagents in the *Quick*-RNA™ Viral 96 Kit (i.e., batch 0, batch 1, batch 2, batch 3). All batches were manufactured at different time points. All batches were tested, each in triplicate preps, for the extraction of a standard viral RNA control diluted in plasma and quantified by RT-qPCR according to quality control, standard operating procedure (R1040-E/R1041-E). For inter-assay reproducibility, the 4 batches manufactured at different periods of time show similar results in mean value and standard deviation (table 2).

Table 1. Intra-assay (repeatability)

	Batch 0 (test 1)		**Batch 0 (test 2)**		**Batch 0 (test 3)**		**Global**		NTC (n=3)
viral dilution	1X	10X	1X	10X	1X	10X	**1X**	**10X**	
rep. 1	27.32	30.45	27.3	30.71	27.34	30.73			
rep. 2	27.8	30.22	27.34	30.73	27.23	30.48			
rep. 3	27.12	30.28	27.17	30.48	26.96	30.9			
avg. C(t)	27.41	30.32	27.27	30.64	27.18	30.70	**27.29**	**30.55**	no signal
stdev	0.35	0.12	0.09	0.14	0.20	0.21	**0.12**	**0.20**	

Table 2. Inter-assay (intermediate fidelity)

	Batch 0		**Batch 1**		**Batch 2**		**Batch 3**		NTC (n=3)
viral dilution	1X	10X	1X	10X	1X	10X	1X	10X	
rep. 1	27.32	30.45	27.61	30.66	27.14	30.78	27.21	30.84	
rep. 2	27.8	30.22	27.35	30.54	27.24	30.5	27.16	30.69	
rep. 3	27.12	30.28	27.15	31.01	27.28	30.41	27.31	30.48	
avg. C(t)	27.41	30.32	27.37	30.74	27.22	30.56	27.23	30.67	no signal
stdev	0.35	0.12	0.23	0.24	0.07	0.19	0.08	0.18	

	Global	
viral dilution	**1X**	**10X**
rep. 1		
rep. 2		
rep. 3		
avg. C(t)	**27.31**	**30.57**
stdev	**0.20**	**0.23**

Ordering Information

Product Description	Catalog No.	Size
***Quick*-RNA™ Viral 96 Kit**	R1040-E R1041-E	2 x 96 preps. 4 x 96 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 I-96 Plate	C2004	2
Collection Plate	C2002	2
Elution Plate	C2003	2
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

Symbols

	Authorized representative in the European community/European Union
	CE IVD vertical
	CE IVD horizontal
	Warning
	*In vitro* diagnostic medical device
	Lot number
	Manufacturer
	Reference **or** Catalogue number
	Consult instructions for use
	Temperature limit
	Unique device identifier
	Use-by-date
	Contains sufficient for < n > preps or reactions

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 or perform DNase I treatment post-purification (#R1080), then clean-up the treated sample.

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

Notes

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Zymo Research is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, please call 1(888) 882-9682.

Integrity of kit components is guaranteed for up to one year from date of purchase.
Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product should only be used by trained professionals. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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