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 or **REF**
R1034-E, R1035-E

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

CE IVD

For *in vitro* diagnostic

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Table of Contents

Product Contents

Quick-RNA™ Viral Kit	R1034-E (50 prep)	R1035-E (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.
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**. Store at room temperature.
2 Add 24 ml of 100% ethanol (26 ml of 95% ethanol) to the 6 ml **Viral Wash Buffer** concentrate (R1034-E) or 192 ml of 100% ethanol (204 ml of 95% ethanol) to the 48 ml **Viral Wash Buffer** concentrate (R1035-E). Store at room temperature.

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 6.

- **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) **–**

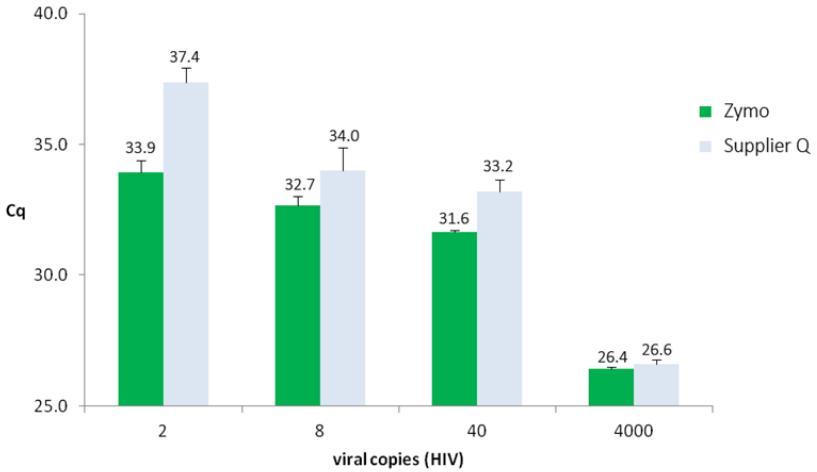
  DNase I Set (E1010; 50 rxns.; 250 U DNase I (lyophilized) supplied w/ DNA Digestion Buffer, 4 ml)
  RNA Prep Buffer (R1060-2-50; 50 ml)
  RNA Wash Buffer (concentrate) (R1003-3-6, 6 ml)
  Proteinase K Set (D3001-2-20; 20 mg Proteinase K (lyophilized) supplied w/ Storage Buffer).

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.

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.

- ✓ 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)).
- ✓ Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and European regulations.
- ✓ The following warning apply:

GHS07

Acute Tox. 4 H302 Harmful if swallowed.

Skin Irrit. 2 H315 Causes skin irritation.

Eye Irrit. 2B H320 Causes eye irritation.

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 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-E) or 192 ml of 100% ethanol (204 ml of 95% ethanol) to the 48 ml **Viral Wash Buffer** concentrate (R1035-E).

(II) Sample Preparation

- ✓ Perform all steps at room temperature (20-30°C).
- ✓ Up to 400 µl sample can be processed per prep.

Samples in DNA/RNA Shield™[1] collection devices (swabs, saliva, etc.)
Proceed directly with purification, page 7.

Swabs (UTM®/VTM®, PBS, saline, etc.)
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 an equal volume of **DNA/RNA Shield™** (2X concentrate) to a volume of liquid sample (1:1) and mix well. 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 (4-25°C) for a month, 3 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 8).
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 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 7, 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 (20-30°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 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 24 ml of 100% ethanol (26 ml of 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate.

Performance

Repeatability

Repeatability was performed with 1 specific manufactured lot of reagents in the *Quick*-RNA™ Viral 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 (R1034-E/R1035-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 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 (R1034-E/R1035-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	27.20	30.60	27.15	30.73	27.16	30.85			
rep. 2	27.17	30.52	27.23	30.48	27.31	30.84			
rep. 3	27.20	30.50	27.12	30.63	27.22	30.69			
avg. C(t)	27.19	30.54	27.17	30.61	27.23	30.79	**27.20**	**30.65**	no signal
stdev	0.02	0.05	0.06	0.13	0.08	0.09	**0.06**	**0.14**	

Table 2. Inter-assay (intermediate fidelity)

	Batch 0		**Batch 1**		**Batch 2**		**Batch 4**		NTC (n=3)
viral dilution	1X	10X	1X	10X	1X	10X	1X	10X	
rep. 1	27.20	30.60	27.28	31.04	27.21	30.55	27.23	30.65	
rep. 2	27.17	30.52	27.34	31.04	27.16	30.82	27.45	30.73	
rep. 3	27.20	30.50	27.22	30.79	27.14	30.81	27.49	30.6	
avg. C(t)	27.19	30.54	27.28	30.96	27.17	30.73	27.39	30.66	no signal
stdev	0.02	0.05	0.06	0.14	0.04	0.15	0.14	0.07	

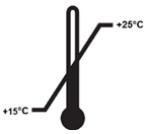
	Global	
viral dilution	**1X**	**10X**
rep. 1		
rep. 2		
rep. 3		
avg. C(t)	**27.26**	**30.72**
stdev	**0.11**	**0.19**

Ordering Information

Product Description	Catalog No.	Size
***Quick*-RNA™ Viral Kit**	R1034-E R1035-E	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

Authorized representative:
EC REP
Catherine David,
42 route du périmètre,
74940 Annecy, France



Symbols

Reference

Number of reactions

Storage temperature

Manufacturer

Expiry date

Lot number

Harmful

See instruction manual

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 6). 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 8) 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

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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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