



Quick-RNA[™] Viral 96 Kit

Viral RNA from any biological sample

Highlights

- Spin-plate (96-well) purification of viral RNA from plasma, serum, ٠ 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, R1041



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







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Product Contents

| Quick-RNA [™] Viral 96 Kit | R1040 (2 x 96 prep) | R1041 (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.

- 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) -

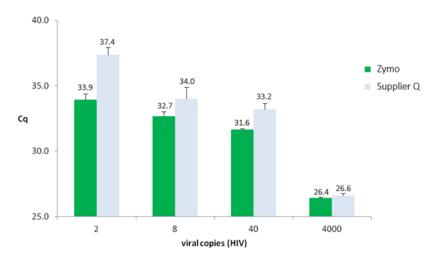
DNase I Set (E1010; 50 rxns.; 250 U DNase I (lyophilized) supplied w/ DNA Digestion Buffer, 4 ml) RNA Prep Buffer (R1060-2-50; 50 ml) RNA Wash Buffer (concentrate) (R1003-3-6, 6 ml) Proteinase K Set (D3001-2-20; 20 mg Proteinase K (lyophilized) supplied w/ Storage Buffer).

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.

Protocol

The protocol consists of: (I) Buffer Preparation, (II) Sample Preparation and (III) DNA/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 (20-30°C).
- \checkmark Up to 400 µl sample can be processed per prep.

<u>Samples in DNA/RNA Shield^{™1} collection devices</u> (swabs, saliva, etc.) Proceed directly with purification, page 6.

Swabs (UTM[®]/VTM[®], PBS, saline, etc.)

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.

<u>Liquids</u> (plasma², serum², 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 6.

<u>Tissue</u>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 μ l Proteinase K to each 400 μ l sample.

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 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 sample¹ (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 7).

- Add 500 µl Viral Wash Buffer to each well, centrifuge and discard the flow-through. <u>Repeat this step</u>.
- 4. Add 500 μl ethanol (95-100%) to each well and centrifuge. Then mount the spin-plate onto an **Elution Plate**.
- 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 \geq 10 µl elution.

The eluted RNA³ 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 can be processed per prep.

² To process > 700 μ l, 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) (R1003-3-6); 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/uI) ^{1,2} | 5 µl |

- 1. Following RNA binding (page 6, 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 (20-30°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 6, step 3).

3 Before use, add 24 ml of 100% ethanol (26 ml of 95% ethanol) to the 6 ml RNA Wash Buffer concentrate.

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.

Ordering Information

| Product Description | Catalog No. | Size |
|-------------------------------------|----------------|--------------------------------|
| Quick-RNA [™] Viral 96 Kit | R1040 R1041 | 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 |

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 (#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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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.

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