ZYMO RESEARCH

RNA Purification Made Simple™

Quick-DNA/RNA™ Viral Kit

Viral DNA & RNA from any biological sample

Highlights

- Quick, spin-column purification of viral DNA and RNA from plasma, serum, urine, cell culture media, blood, saliva, cellular suspensions, swab, fecal and biopsy samples
- High-quality DNA/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:
D7020, D7021

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

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Table of Contents

Product Contents

Quick-DNA/RNA™ Viral Kit	D7020 (50 prep)	D7021 (200 prep)
DNA/RNA Shield™ (2X concentrate)	25 ml	125 ml
Viral DNA/RNA Buffer[1]	25 ml (x2)	100 ml (x2)
Viral Wash Buffer[2] (concentrate)	6 ml (x2)	24 ml (x2)
DNase/RNase-Free Water	6 ml	6 ml (x2)
Zymo-Spin™ IIC-XLR 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 125 µl or 500 µl β-Me per 25 ml or 100 ml **Viral DNA/RNA Buffer.**
2 Add 24 ml of 100% ethanol (26 ml of 95% ethanol) to the 6 ml **Viral Wash Buffer** concentrate (D7020) or 192 ml of 100% ethanol (204 ml of 95% ethanol) to the 48 ml **Viral Wash Buffer** concentrate (D7021).

Specifications

- **Sample Sources –** ≤ 400 µl plasma, serum, saliva, swab, urine, cell culture media, blood, cellular suspension, fecal sample or ≤ 25 mg biopsy sample.

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

- **Purity –** DNA/RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.

- **Binding Capacity –** 50 µg DNA/RNA (**Zymo-Spin™ IIC-XLR Columns**).

- **Elution Volume –** ≥ 50 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)
  DNA/RNA Prep Buffer (D7010-2-50; 50 ml)
  DNA/RNA Wash Buffer (concentrate) (D7010-3-6, 6 ml)
  Proteinase K Set (D3001-2-20; 20 mg Proteinase K (lyophilized) supplied w/ Storage Buffer).

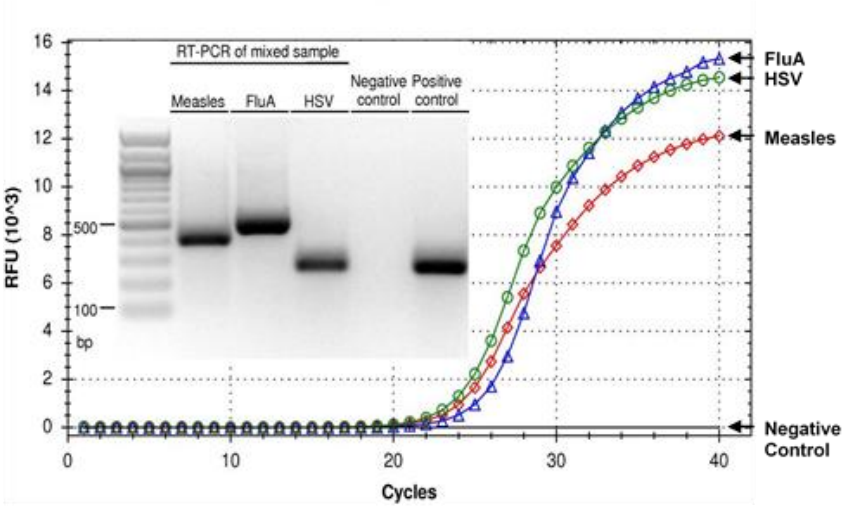
Product Description

The ***Quick*-DNA/RNA™ Viral Kit** is a quick, purification of viral DNA and/or 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 nucleic acids are ready for all downstream applications such as Next-Gen sequencing, hybridization-based and RT/qPCR detection.

Detection of DNA & RNA Viruses from a Mixed Population



Viral nucleic acids were isolated from liquid samples using the ***Quick*-DNA/RNA™ Viral Kit**. Data shows RT-qPCR Ct values for measles, influenza type A (FluA), and herpes-simplex (HSV) viruses, 23.05 (diamonds), 24.56 (triangles), 22.92 (circles), respectively. Negative control – RT-PCR (no template w/ HSV specific primers). Positive control – PCR (HSV template w/ HSV primers).

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 125 µl or 500 µl β-Me per 25 ml or 100 ml **Viral DNA/RNA Buffer.**
- ✓ Add 24 ml of 100% ethanol (26 ml of 95% ethanol) to the 6 ml **Viral Wash Buffer** concentrate (D7020) or 96 ml of 100% ethanol (104 ml of 95% ethanol) to the 24 ml **Viral Wash Buffer** concentrate (D7021).

(II) Sample Preparation

- ✓ Perform all steps at room temperature (20-30°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 25 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 (4-25°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) DNA/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 DNA/RNA Buffer** to each 400 µl sample[1] (2:1) and mix well.
2. Transfer the mixture into a **Zymo-Spin™ IIC-XLR 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. Add 50 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge for 30 seconds.

   Alternatively, for highly concentrated DNA/RNA use ≥ 35 µl elution.

   The eluted DNA/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 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), DNA/RNA Prep Buffer (D7010-2-50) and DNA/RNA Wash Buffer (concentrate) (D7010-3-6); 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	75 µl
DNase I (reconstituted; 1 U/ul)[1,2]	5 µl

1. Following DNA/RNA binding (page 6, step 2), add 400 µl **DNA/RNA Wash Buffer**[3] to the column, centrifuge and discard the flow-through.
2. Add 80 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 **DNA/RNA Prep Buffer** to the column, centrifuge and discard the flow-through.
5. Proceed with DNA/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 **DNA/RNA Wash Buffer** concentrate.

Ordering Information

Product Description	Catalog No.	Size
***Quick*-DNA/RNA™ Viral Kit**	D7020 D7021	50 preps. 200 preps.

Individual Kit Components	Catalog No.	Amount
DNA/RNA Shield™ (2X concentrate)	R1200-25 R1200-125	25 ml 125 ml
Viral DNA/RNA Buffer	D7020-1-25 D7020-1-100	25 ml 100 ml
Viral Wash Buffer (concentrate)	R1034-2-24 R1034-2-48	24 ml 48 ml
Zymo-Spin™ IIC-XLR	C1104-25 C1104-50	25 50
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
DNA/RNA Prep Buffer	D7010-2-50 D7010-2-200	50 ml 200 ml
DNA/RNA Wash Buffer	D7010-3-6 D7010-3-24	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 (#R1017), 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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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 is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. 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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