ZYMO RESEARCH

RNA Purification Made Simple™

Quick-DNA/RNA™ Pathogen Miniprep

DNA & RNA from any vector-borne pathogen

Highlights

- Spin-column purification of pathogen (virus, bacteria, protozoa) DNA and RNA from a wide variety of vectors (mosquitoes, fleas, ticks, etc.) and tissue types (mammals, birds, etc.).
- 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:
R1042, R1043

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

Table of Contents

Product Contents

Quick-DNA/RNA™ Pathogen Miniprep	R1042 (50 prep)	R1043 (200 prep)
DNA/RNA Shield™	50 ml	250 ml
Pathogen DNA/RNA Buffer[1]	50 ml	100 ml (x2)
Proteinase K[2] (lyophilized) w/ Storage Buffer	5 mg	20 mg
Zymo-Spin™ IICR Columns	50	200
Collection Tubes	50	200
Pathogen DNA/RNA Wash Buffer[3] (concentrate)	6 ml (x2)	48 ml
ZymoBIOMICS™ DNase/RNase-Free Water	3 ml	10 ml (x2)
Instruction Manual	1 pc	1 pc
ZR Bashing Bead™ Lysis Tubes (sold separately)	S6014-50 (50 pack)	

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Add beta-mercaptoethanol to 0.5% (v/v) *i.e.,* add 250 µl or 500 µl β-Me per 50 ml or 100 ml **Pathogen DNA/RNA Buffer.**
2 Reconstitute lyophilized **Proteinase K** according to page 4, Buffer Preparation. Store frozen aliquots.
3 Add 24 ml of 100% ethanol (26 ml of 95% ethanol) to the 6 ml **Pathogen DNA/RNA Wash Buffer** concentrate (R1042) or 192 ml of 100% ethanol (204 ml of 95% ethanol) to the 48 ml **Pathogen DNA/RNA Wash Buffer** concentrate (R1043).

Specifications

- **Sample Sources –** ≤ 10 mg vectors (mosquitoes, fleas, ticks, other tough-to-lyse insects) and tissue types (animal tissue, plants, other hosts) or up to 400 µl liquid sample (e.g., samples in DNA/RNA Shield™).

  For samples in UTM®/VTM®, PBS or saline, see Sample Preparation, page 6.

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

- **Binding Capacity –** 50 µg DNA/RNA **Zymo-Spin™ IICR Columns**.

- **Elution Volume –** ≥ 25 µl **ZymoBIOMICS™ DNase/RNase-Free Water**.

- **Equipment Needed** (user provided) **–** Beta-mercaptoethanol (b-Me), Ethanol (95-100%), Microcentrifuge.

- **Materials** (available separately) **–**

  ZR BashingBead™ Lysis Tubes (S6014-50; 0.1 & 2.0 mm beads)
  DNA/RNA Shield™ (R1200; 2X concentrate)
  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)

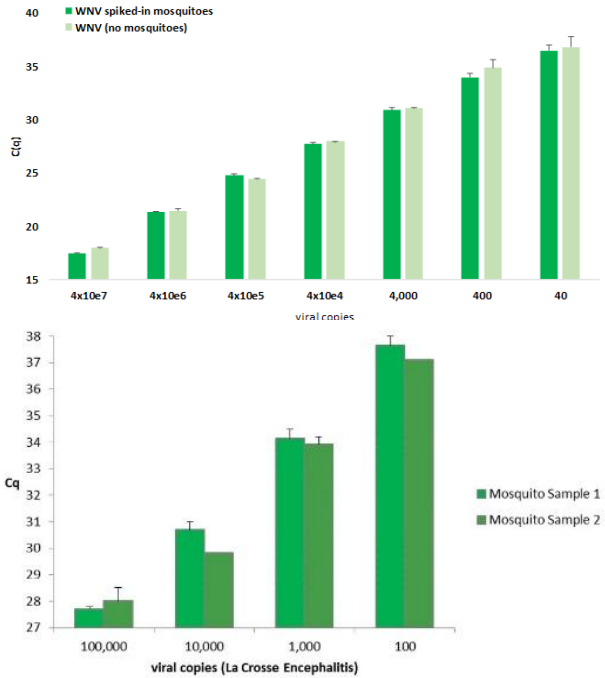
Product Description

The ***Quick*-DNA/RNA™ Pathogen Miniprep** kit is a spin-column purification of pathogen (virus, bacteria, protozoa) DNA and RNA from a wide variety of vectors (mosquitoes, fleas, ticks, etc.) and tissue types (mammals, birds, etc.) collected, transported and stored in **DNA/RNA Shield™**. DNA/RNA Shield™ is used for nucleic acid preservation and inactivation of pathogens.

The kit features a storage/lysis buffer system and can be combined with high density ZR BashingBead™ Lysis Tubes (*recommended) to facilitate complete homogenization of hard-to-lyse samples for efficient nucleic acid isolation. Small (>50 nt) and large (> 200 kb) DNA and RNA are bound to the column, washed and then eluted.

The isolated high-quality nucleic acids are suitable for all downstream applications such as Next-Gen sequencing, hybridization-based and RT/qPCR detection.

Inhibitor-free Detection of Viruses in Mosquitoes



West Nile Virus (top) and La Crosse Encephalitis (bottom) viral particles were spiked-in mosquito homogenate, purified using the ***Quick*-DNA/RNA™ Pathogen** kit and then detected by RT-qPCR.

Workflow

Pathogen
virus, bacteria, protozoa

Vectors
insects, mammals, birds, etc.

Biological samples
biopsies, fecal, blood, etc.

DNA/RNA Shield™
add to sample for collection, transport and storage

*recommended:
Bashing Bead Lysis

Spin-column
catalog no. R1042, R1043

MagBead
catalog no. R2145, R2146

Bind, Wash and Elute!

DNA/RNA is ready for
NGS, (RT)PCR, etc.

Protocol

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

(I) Buffer Preparation

- ✓ Add beta-mercaptoethanol to 0.5% (v/v) i.e., add 250 µl or 500 µl β-Me per 50 ml or 100 ml **Pathogen DNA/RNA Buffer**, respectively.
- ✓ Add 24 ml of 100% ethanol (26 ml of 95% ethanol) to the 6 ml **Pathogen DNA/RNA Wash Buffer** concentrate (R1042) or 192 ml of 100% ethanol (204 ml of 95% ethanol) to the 48 ml **Pathogen DNA/RNA Wash Buffer** concentrate (R1043).
- ✓ Add 260 µl or 1,040 µl **Proteinase K Storage Buffer** to reconstitute per 5 mg or 20 mg lyophilized **Proteinase K**, respectively. Mix by vortexing. Use immediately or store frozen aliquots.

(II) Sample Preparation

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

Tissue[1] (tough-to-lyse insects and tissue)

Up to 10 mg tissue (see table below) can be homogenized in ≥ 400 µl **DNA/RNA Shield™**[2] with a mortar/pestle, dounce, syringe, tissue grinder, or bead beating with a high-speed homogenizer. To remove particulate debris, centrifuge the homogenate at 10,000-16,000 x g for 1 minute, and transfer the cleared supernatant into a nuclease-free tube. Proceed with purification, page 7.

Recommended: To a ZR BashingBead™ Lysis Tube (#S6014; sold separately), add the appropriate amount of sample to **DNA/RNA Shield™**, then mechanically homogenize[3].

Input	Mosquitoes	Ticks	Fleas	Deer fly	Tissue
per prep (pool)	50	1 engorged 5 flat adults 20 nymphs	10	1 adult	animal, plant (≤ 10 mg)
High-speed[3]	3-5 minutes				30-60 seconds
Low-speed[3]	≥ 10 minutes (optimization may be required)				

Swabs (UTM®/VTM®, PBS, saline, etc.)

Proceed directly with purification, page 7.

Optional: To inactivate, store and preserve samples at room temperature prior to processing, add an equal volume of DNA/RNA Shield™ (2X concentrate) (#R1200; sold separately) to a volume of liquid sample (1:1) and mix well.

Samples in DNA/RNA Shield™[2] collection devices (swabs, saliva, etc.)

Proceed directly with purification, page 7.

Optional - **Proteinase K treatment** (protein-rich samples e.g., tissue and biological liquids, can be treated):

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 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).
2 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.
3 Perform bead beating at maximum speed with a high-speed homogenizer (e.g., MP Bio FastPrep-24, Bertin Precellys, etc.) or with a low-speed homogenizer (e.g., Vortex Genie, etc.).

(III) DNA/RNA Purification

- ✓ Perform all steps at room temperature (20-30°C) and centrifuge at 10,000-16,000 x g for 30 seconds, unless specified.
- ✓ The sample input can be scaled up or down, proportionally.

1. Add 800 µl **Pathogen DNA/RNA Buffer**[1] to each 400 µl sample[2] (2:1) and mix well[2].

2. Transfer the mixture into a **Zymo-Spin™ IICR Column**[3] in a **Collection Tube** and centrifuge. Discard the flow-through.

   Optional: At this point, DNase I treatment can be performed (see Appendices, page 8).

3. Add 500 µl **Pathogen DNA/RNA Wash Buffer** to the column and centrifuge. Discard the flow-through, repeat this step.

4. Add 500 µl ethanol (95-100%) to the column and centrifuge for 1 minute to ensure removal of any residual ethanol. Discard the collection tube and carefully transfer the column into a new nuclease-free tube (not provided).

5. To elute DNA/RNA, add 50 µl **ZymoBIOMICS™ DNase/RNase-Free Water** directly to the column matrix and centrifuge.

   Alternatively, for highly concentrated DNA/RNA use ≥ 25 µl elution volume.

   The eluted DNA/RNA[4] can be used immediately or stored frozen.

1 To ensure efficient lysis and deproteinization, up to 5 volumes of Pathogen DNA/RNA Buffer can be used per volume of sample.
2 Up to 400 µl sample can be processed per prep.
3 For volumes > 700 µl, column can be reloaded.
4 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.
- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

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 7, 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 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 **DNA/RNA Wash Buffer** concentrate.

Ordering Information

Product Description	Catalog No.	Size
***Quick*-DNA/RNA™ Pathogen Miniprep**	R1042 R1043	50 preps. 200 preps.

Individual Kit Components	Catalog No.	Amount
Pathogen DNA/RNA Buffer	R1042-1-50 R1042-1-100	50 ml 100 ml
Proteinase K Set supplied w/ Storage Buffer	D3001-2-5 D3001-2-20	5 mg 20 mg
Zymo-Spin™ IICR Columns	C1078-50 C1078-250	50 250
Collection Tubes (2 ml)	C1001-50 C1001-500	50 500
Pathogen DNA/RNA Wash Buffer (concentrate)	R1042-2-6 R1042-2-48	6 ml 48 ml
ZymoBIOMICS™ DNase/RNase-Free Water	D4302-5-3 D4302-5-10	3 ml 10 ml
DNA/RNA Shield™	R1100-50 R1100-250	50 ml 250 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	50 ml

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 (tissue, biological liquids, etc.): - Increase the volume of DNA/RNA Shield™ added to the sample. - Perform Proteinase K treatment (see Sample Preparation, page 5). 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 (#R1017), 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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