

INSTRUCTION MANUAL

Quick-DNA/RNA™ Pathogen Miniprep

Catalog Nos. R1042 & R1043

Highlights

- Spin-column purification of pathogen (virus, bacteria, protozoa) DNA/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/PCR, hybridization, etc.
- DNA/RNA Shield[™] for sample collection, inactivation and storage provided.

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product, please call 1-888-882-9682.

Product Contents:

Quick-DNA/RNA Pathogen Miniprep (Kit Size)	R1042 (50 preps)	R1043 (200 preps)	Storage Temperature
DNA/RNA Shield™	50 ml	250 ml	Room Temp.
Pathogen DNA/RNA Buffer ¹	50 ml	100 ml	Room Temp.
Proteinase K w/ Storage Buffer ²	5 mg	20 mg	Room Temp.
Zymo-Spin [™] IICR Columns	50	200	Room Temp.
Collection Tubes	50	200	Room Temp.
Pathogen DNA/RNA Wash Buffer (concentrate) ³	2x 6 ml	48 ml	Room Temp.
DNase/RNase-Free Water	4 ml	2x 10 ml	Room Temp.
Instruction Manual	1	1	Room Temp.
ZR Bashing Bead [™] Lysis Tubes (sold separately)	S6014-50 (1x 50 pag	k or 4x 50 pack)	Room Temp.

Note - 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 maximal performance and reliability.

Specifications:

- **Sample Type:** ≤10 mg vectors (mosquitoes, fleas, ticks, other tough-to-lyse insects) and tissue types (animal tissue, plants, other hosts) or ≤200 µl biological liquids.
- **Purity:** High-quality DNA/RNA ready for Next-Gen sequencing, RT/PCR, hybridization, *etc.*
- Binding Capacity: 50 µg DNA/RNA (Zymo-Spin™ IICR Columns)
- Size: 50 nt to ~200 kb
- Elution Volume: ≥25 µl with DNase/RNase-Free Water
- Materials (avaliable separately):
 ZR BashingBead Lysis Tubes (S6014; 0.1/2.0 mm)

Notes:

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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 $^{^1}$ Add beta-mercaptoethanol to 0.5% (v/v) *i.e.*, add 250 μ l or 500 μ l per 50 ml or 100 ml **Pathogen DNA/RNA Buffer**, respectively.

² Add 260 μl or 1,040 μl **Proteinase K Storage Buffer** to reconstitute per 5 mg or 20 mg lyophilized **Proteinase K**, respectively. Vortex to dissolve and 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) before use.

Product Description

Quick-DNA/RNA™ Pathogen Miniprep kit is designed for high-throughput 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™**.

The kit features a storage/lysis buffer system and can be combined with high density ZR BashingBead™ Lysis Tubes (*optional) 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 **Zymo-Spin™ IICR Columns**, 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/PCR detection.

For **Technical Assistance**, please contact **Zymo** at 1-888-882-9682 or E-mail tech@zymoresearch.com.

Inhibitor-free detection of West Nile Virus in mosquitoes

DNA/RNA Shield™

Pathogen

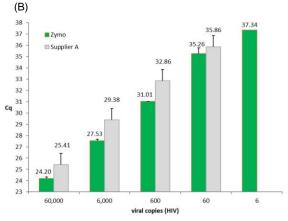
virus, bacteria, protozoa

Vectors
insects, mammals, birds, etc.
Biological samples
biopsies. fecal, blood, etc.

add to sample for collection, transport and storage



High-sensitivity detection of HIV-1 virus at low titer



(A) West Nile Virus (spiked-in mosquito homogenate),
 (B) HIV-1 viral RNA particles (spiked-in plasma),
 purified using the *Quick-DNA/RNA™* Pathogen kit and detected by RT-qPCR.



Reagent Preparation:

- ✓ Add 500 µl or 1 ml beta-mercaptoethanol (user supplied) per 100 ml or 200 ml **Pathogen DNA/RNA Buffer**, respectively (final concentration 0.5% (v/v)).
- ✓ Add 20 mL (R2145) or 80 ml (R2146) of isopropanol to the MagBead DNA/RNA Wash 1 concentrate.
- 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) before use.

Protocol:

The following procedure should be performed at room temperature (15-30°C), unless specified.

Sample Preparation

1. Add **DNA/RNA Shield**[™] to the sample as recommended (table below) in a nuclease-free tube (not provided) and mix well:

		add DNA/RNA Shield ™
		#R1100
Insects ¹ (tough-to-lyse, ticks, mosquitoes, fleas, deer flies, etc.)	<10 mg	900
Tissue	≤10 mg	800 µl
(mammals, birds, plants)		

For biological samples² collected and stored in DNA/RNA Shield™ (i.e., #R1200, R1101, R1150, R1104, R1107, R1109, etc.), proceed to step 3 below.

 Optional: Mechanical homogenization with the ZR BashingBead[™] Lysis Tube³ and a high-speed cell disruptor⁴ is recommended for tough-to-lyse insects, animal tissue, plants, etc.

	homogenization time (high-speed)
insects (tough-to-lyse, ticks, mosquitoes, fleas, deer flies, etc.)	3-5 minutes
tissue (mammals, birds, plants)	30-60 seconds

3. To remove particulate debris or precipitation, centrifuge at 10,000-16,000 x g for 1 minute. Transfer up to 200 μl of the cleared supernatant⁵ into a nuclease-free tube (not provided).

Notes:

- ¹ See Appendix, page 5 for specific number of insect input.
- ² Plasma, serum, wholeblood, urine, fecal, swab, saliva, cell suspension, culture media, etc.
- ³ Catalog no. 6014-50
- ⁴ Required homogenization time will vary depending on the device and application. For high-speed cell disruptors (e.g., FastPrep® 24, TerraLyzer™ Sample Processor or similar), samples can be processed in ≤5 minutes. For low-speed cell disruptors (e.g., Disruptor Genie™, or standard benchtop vortexes), processing can be ≤20 minutes long. See manufacturer's literature for operating information.
- ⁵ Up to 200 μl liquid sample can be processed per prep.

DNA/RNA Purification

- Perform all steps at room temperature and centrifugation 10,000-16,000 x g for 30 seconds, unless specified.
- 1. Add 2 µl **Proteinase K** and mix well.
- 2. Add 400 μl **Pathogen DNA/RNA Buffer**⁶ to each 200 μl sample⁵, mix well and incubate at room temperature for 5 minutes.
- 3. Transfer the mixture into a **Zymo-Spin**[™] **IICR Column**⁷ in a **Collection Tube** and centrifuge. Discard the flow-through.
- 4. Add 500 µl **Pathogen DNA/RNA Wash Buffer**⁸ to the column and centrifuge. Discard the flow-through and repeat this step.
- 5. 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).
- 6. Add 50 μl **DNase/RNase-Free Water** directly to the matrix of the column and centrifuge to elute the DNA/RNA.

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

The eluted DNA/RNA⁹ can be used immediately or stored frozen.

Notes:

- ⁵ Up to 200 μl liquid sample can be processed per prep.
- ⁶ To ensure efficient lysis and deproteinization, up to 5 volumes of Pathogen DNA/RNA Buffer can be used per 200 μl liquid sample.
- ⁷ Volume capacity of the column is 800 μl.
- ⁸ Before starting, add the appropriate amount of ethanol to the wash buffer concentrate, see Buffer Preparation, page 3.
- ⁹ It is recommended to titrate the DNA/RNA eluate for downstream applications (i.e., RT/PCR, etc.).

Appendix:

Insect Samples: Recommended Input

Sample type	Maximum specimen input per 800 µl DNA/RNA Shield™	
mosquito	≤ 50	
tick	1 engorged of any species ≤ 5 flat adults ≤ 20 nymphs	
flea	≤ 10	
deer fly	1 adult	

Ordering Information:

Product Description	Kit Size	Catalog No.
<i>Quick</i> -DNA/RNA [™] Pathogen Miniprep	R1042 R1043	50 Preps 200 Preps
<i>Quick</i> -DNA/RNA [™] Pathogen MagBead	R2145 R2146	96 Preps 4x 96 Preps

For Individual Sale	Amount	Catalog No.
DNA/RNA Shield™	50 ml 250 ml	R1100-50 R1100-250
Pathogen DNA/RNA Buffer	50 ml 100 ml	R1042-1-50 R1042-1-100
Proteinase K w/ Storage Buffer Set	5 mg 20 mg	D3001-2-5 D3001-2-20
Zymo-Spin [™] IICR Columns	C1078-50 C1078-250	50 pack 200 pack
Collection Tubes (2 ml)	C1001-50 C1001-500	50 pack 500 pack
Pathogen DNA/RNA Wash Buffer (concentrate)	R1042-2-6 R1042-2-48	12 ml 48 ml
DNase/RNase-Free Water	30 ml 100 ml 200 ml	W1001-30 W1001-100 W1001-200

