ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

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*.) stored in DNA/RNA Shield™
- DNA/RNA is ready for any sensitive downstream applications (e.g., Next-Gen sequencing, RT/PCR, *etc.*)

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ZYMO RESEARCH CORP.

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product please contact us.

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 the highest performance and reliability.

Product Contents

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

Storage Temperature – Store all kit components (*i.e.,* buffers, columns) at room temperature.
[1] Add beta-mercaptoethanol to 0.5% (v/v) *i.e.,* add 250 µl or 500 µl per 50 ml or 100 ml **Pathogen DNA/RNA Buffer**, respectively.
[2] Add 260 µl or 1,040 µl **Proteinase K Storage Buffer** per vial to reconstitute the lyophilized **Proteinase K,** 5 mg or 20 mg 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.

Specifications

- **Sample Sources** – vectors (mosquitoes, fleas, ticks, other tough-to-lyse insects) and tissue types (animal tissue, plants, other hosts) processed and stored in DNA/RNA Shield™.
- **Sample Size** – ≤200 µl (or ≤10 mg).
- **Format** – 50 µg DNA/RNA binding capacity (spin-column), ≥25 µl elution volume
- **Size Range** – 50 nt to ~200 kb
- **Purity** – DNA/RNA is ready for any sensitive downstream applications (e.g., Next-Gen sequencing, RT/PCR, *etc.*)
- **Materials** (sold separately) –

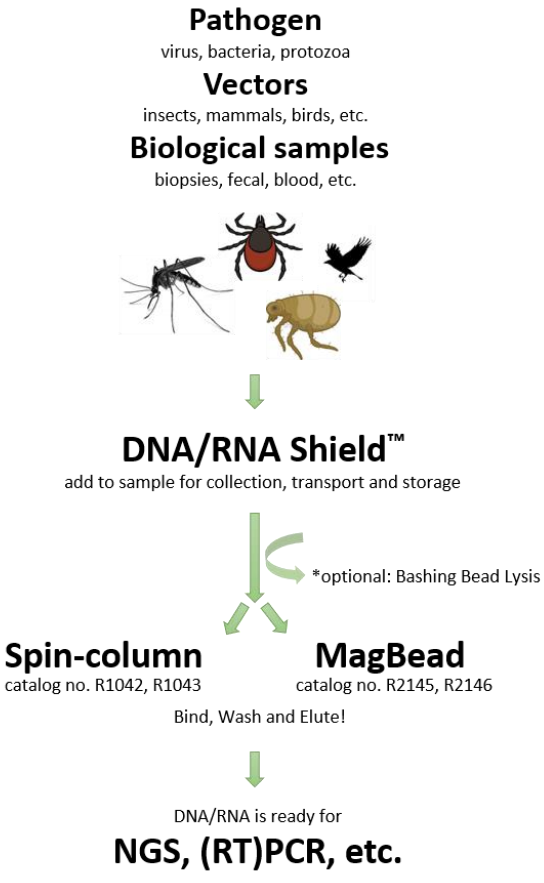
  ZR BashingBead Lysis Tubes; (S6014; 0.1/2.0 mm)

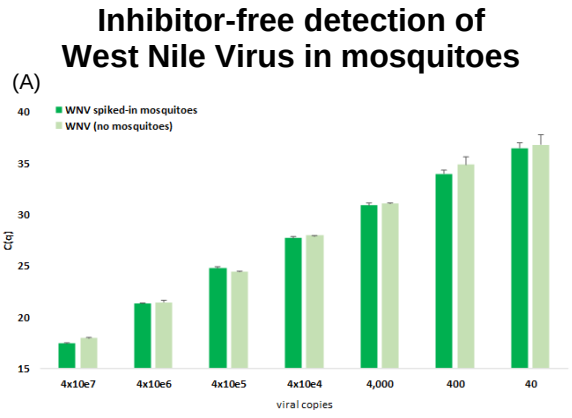
Product Description

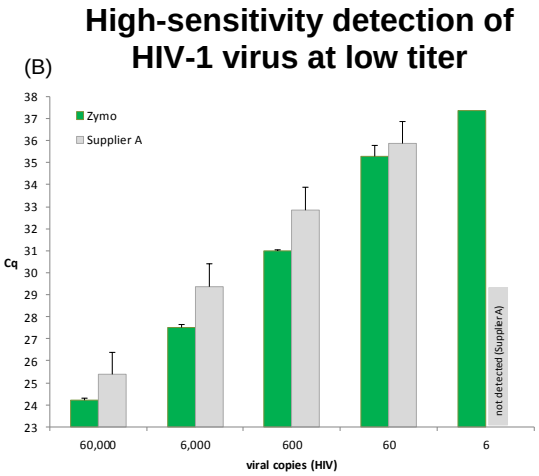
***Quick*-DNA/RNA™ Pathogen Miniprep** kit is a spin-column based purification of pathogen (virus, bacteria, protozoa) DNA and RNA from a wide variety of vectors (mosquitoes, fleas, ticks, *etc*.) and tissue types (mammals, birds, *etc*.) collected, transported and stored in **DNA/RNA Shield™**.

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™ IIC 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.







(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.

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

***optional**

ZR BashingBead Lysis Tubes 2.0 mm + 0.1 mm (catalog no. S6014)

All steps should be performed at room temperature unless specified.

Buffer Preparation

- Add beta-mercaptoethanol 250 µl or 500 µl per 50 ml or 100 ml **Pathogen DNA/RNA Buffer**, respectively. (final concentration 0.5% (v/v)).
- 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.
- Add 260 µl or 1,040 µl **Proteinase K Storage Buffer** per vial to reconstitute the lyophilized **Proteinase K,** 5 mg or 20 mg respectively. Vortex to dissolve and store frozen aliquots.

Protocol

- All steps should be performed at room temperature, 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	800 µl
Tissue (mammals, birds, plants)		

For biological samples[2] collected and stored in DNA/RNA Shield™ (i.e., #R1200, R1101, R1150, R1104, R1107, R1109, etc.), proceed to step 3 below.

2. Optional: Mechanical homogenization with the ZR BashingBead™ Lysis Tube[3] and a high-speed cell disruptor[4] 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 supernatant[5] into a nuclease-free tube or well/plate (not provided).

Notes:

[1] See Appendices, page 5 for specific number of insect input.

[2] Plasma, serum, whole-blood, urine, fecal, swab, saliva, cell suspension, culture media, etc.

[3] S6014-50, S6014-200

[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.

4. Add 2 µl **Proteinase K** and mix well.

5. Add 400 µl **Pathogen DNA/RNA Buffer**[6] to each 200 µl sample[5], mix well and incubate at room temperature for 5 minutes.

6. Transfer the mixture into a **Zymo-Spin™ IIC Column**[7] in a **Collection Tube** and centrifuge. Discard the flow-through.

7. Add 500 µl **Pathogen DNA/RNA Wash Buffer**[8] to the column and centrifuge. Discard the flow-through and repeat this step.

8. 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).

9. 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/RNA[9] 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.).

Appendices

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	Catalog No.	Kit Size
***Quick*-DNA/RNA™ Pathogen Miniprep**	R1042 R1043	50 Preps 200 Preps
***Quick*-DNA/RNA™ Pathogen MagBead**	R2145 R2146	96 Preps 4x 96 Preps

For Individual Sale	Catalog No.	Amount
DNA/RNA Shield™	R1100-50 R1100-250	50 ml 250 ml
Pathogen DNA/RNA Buffer	R1042-1-50 R1042-1-100	50 ml 100 ml
Proteinase K w/ Storage Buffer Set	D3001-2-5 D3001-2-20	5 mg 20 mg
Zymo-Spin™ IIC Columns	C1011-50 C1011-250	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	W1001-30 W1001-100 W1001-200	30 ml 100 ml 200 ml
ZR BashingBead™ Lysis Tubes (0.1 mm & 2.0 mm)	S6014-50	50 pack

DNA & RNA
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