ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-DNA/RNA™ Pathogen MagBead

Catalog Nos. **R2145 & R2146**

Highlights

- High-throughput, magnetic-bead based 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.*)

Contents

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 MagBead** (Kit Size)	**R2145** (96 preps)	**R2146** (4x 96 preps)
DNA/RNA Shield™	2x 50 ml	2x 250 ml
Pathogen DNA/RNA Buffer[1]	100 ml	4x 100 ml
Proteinase K w/ Storage Buffer[2]	5 mg	20 mg
MagBinding Beads	3 ml	12 ml
MagBead DNA/RNA Wash 1 (concentrate)[3]	30 ml	120 ml
MagBead DNA/RNA Wash 2 (concentrate)[4]	20 ml	80 ml
DNase/RNase-Free Water	10 ml	2x 30 ml
Instruction Manual	1	1
ZR Bashing Bead™ Lysis Tubes (sold separately)	S6014-50 (1x 50 pack or 8x 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 500 µl or 1 ml per 100 ml or 200 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 20 mL (R2145) or 80 ml (R2146) of isopropanol to the **MagBead DNA/RNA Wash 1** concentrate.
[4] Add 30 mL (R2145) or 120 ml (R2146) of isopropanol to the **MagBead DNA/RNA Wash 2** concentrate.

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** – 10 µg DNA/RNA binding capacity (per 20 µl MagBinding Beads), ≥50 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) –

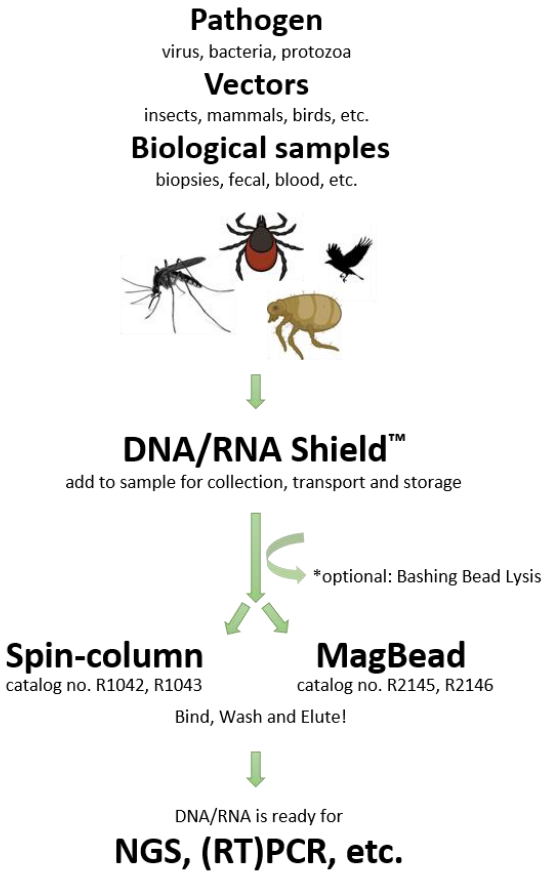
  ZR BashingBead Lysis Tubes (S6014; 0.1/2.0 mm)
  ZR-96 MagStand (P1005)
  Collection Plate (C2002; capacity 1.2 ml/well)
  96-Well Block (P1001; capacity 2 ml/well)
  Elution Plate (C2003; capacity 0.35 ml/well)
  Cover Foil (C2007)

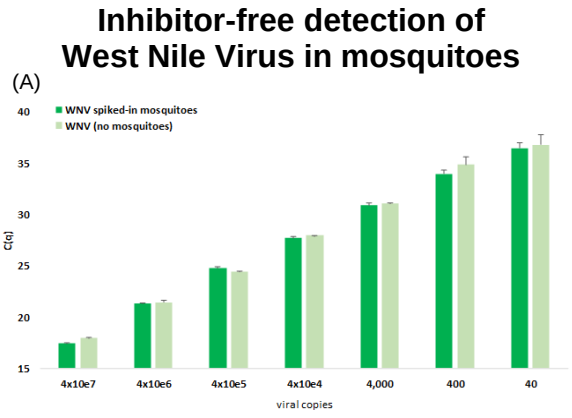
Product Description

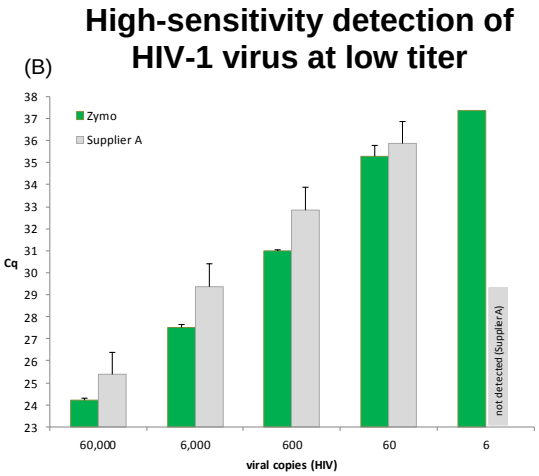
***Quick*-DNA/RNA™ Pathogen MagBead** kit is designed for high-throughput purification of pathogen (virus, bacteria, protozoa) DNA and RNA from a wide variety of vectors (mosquitoes, fleas, ticks, *etc*.) and tissue types (mammals, birds, *etc*.) collected, transported and stored in **DNA/RNA Shield™**.

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 magnetic beads, 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 reagents and steps should be performed at room temperature, unless specified.

Buffer 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 30 mL (R2145) or 120 ml (R2146) of isopropanol to the **MagBead DNA/RNA Wash 2** concentrate.
- Add 260 µl or 1,040 µl **Proteinase K Storage Buffer** per vial to reconstitute the lyophilized **Proteinase K,** 5 mg or 20 mg. 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

4. Add 2 µl **Proteinase K** and mix well.
5. Add 400 µl **Pathogen DNA/RNA Buffer**[6] to each 200 µl sample[7] and mix well[8].
6. Add 20 µl **MagBinding Beads** and mix well[8] for 10 minutes. Important: MagBinding Beads settle quickly, ensure that beads are kept in suspension while dispensing.
7. Transfer the plate to a magnetic stand until beads have pelleted, then aspirate[9] and discard the cleared supernatant.
8. Add 500 µl **MagBead DNA/RNA Wash Buffer 1** and mix well[8].
9. Transfer the plate to a magnetic stand until beads have pelleted, then aspirate[9] and discard the cleared supernatant.
10. Add 500 µl **MagBead DNA/RNA Wash Buffer 2** and mix well[8].
11. Transfer the plate to a magnetic stand until beads have pelleted, then aspirate[9] and discard the cleared supernatant.
12. Add 500 µl ethanol (95-100%) and mix well[8].
13. Transfer the plate to a magnetic stand until beads have pelleted, then aspirate[9] and discard the cleared supernatant.
14. Repeat steps 12 and 13.
15. Dry the beads at room temperature for 10 minutes or until fully dry[10].
16. To elute DNA/RNA from the beads, add 50 µl **DNase/RNase-Free Water** and mix well[8].

    Alternatively, for highly concentrated DNA/RNA use ≥30 µl volume.
17. Transfer the plate to a magnetic stand until beads have pelleted, then aspirate[9] and dispense the eluted DNA/RNA to an elution plate.

    The eluted DNA/RNA[11] can be used immediately or stored frozen.

All reagents and steps should be performed at room temperature, unless specified.

Notes:

[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] Up to 200 µl liquid sample can be processed per prep.

[8] For all buffer additions and to ensure beads are properly in suspension, **mix well** by pipetting up and down several times and/or by shaking (vortexing) at ~1,300 rpm.

[9] Some beads will adhere to the sides of the well. When removing the supernatant, aspirate slowly to allow these beads to be pulled to the magnet as the liquid level is lowered.

[10] Beads will change in appearance from glossy black when still wet to a dull brown when fully dry. Alternatively, a heat block can be used (25-55°C).

[11] 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

Automation Scripts

The ***Quick*-DNA/RNA™ Pathogen MagBead** kit is compatible with any automated platform. For automation scripts and related technical support, email automation@zymoresearch.com. In the subject line, please include "Automation Scripts", instrument used and the product catalog number.

Automation Script for MagMAX Express 24 – AM1836 protocol

Ordering Information

Product Description	Catalog No.	Kit Size
***Quick*-DNA/RNA™ Pathogen MagBead**	R2145 R2146	1x 96 Preps 4x 96 Preps
***Quick*-DNA/RNA™ Pathogen MiniPrep**	R1042 R1043	50 Preps 200 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
MagBinding Beads	D4100-2-3 D4100-2-12	3 ml 12 ml
MagBead DNA/RNA Wash 1 (concentrate)	R2130-1-30 R2130-1-120	30 ml 120 ml
MagBead DNA/RNA Wash 2 (concentrate)	R2130-2-20 R2130-2-80	20 ml 80 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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