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*.).
- High-quality DNA/RNA is ready for Next-Gen sequencing, RT/PCR, hybridization, *etc.*
- DNA/RNA Shield™ for sample collection, inactivation and storage provided.

Contents

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

[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** to reconstitute per 5 mg or 20 mg lyophilized **Proteinase K**, 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 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:** 10 µg DNA/RNA per 20 µl magnetic beads.
- **Size:** 50 nt to ~200 kb
- **Elution Volume:** ≥50 µl ZymoBIOMICS DNase/RNase-Free Water
- **Materials** (avaliable 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; 2, 6, 12 or 24 pack)

Notes:

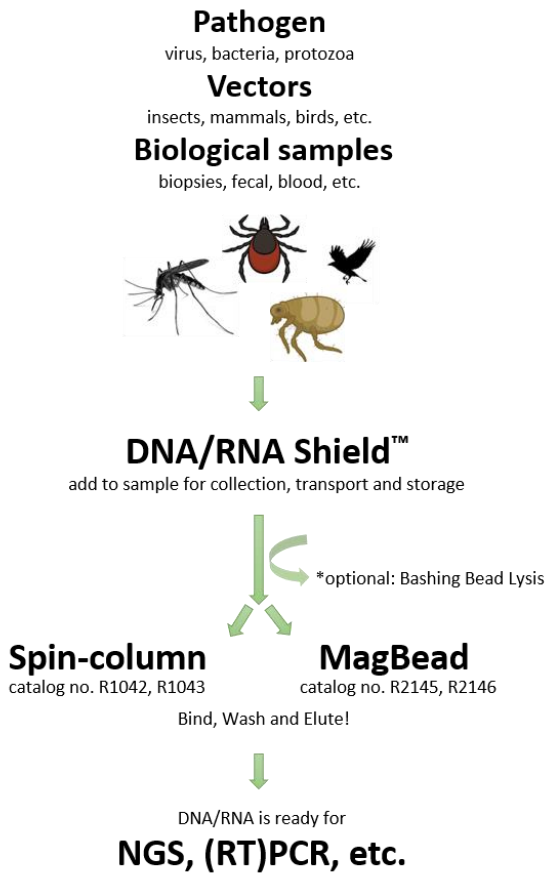
This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

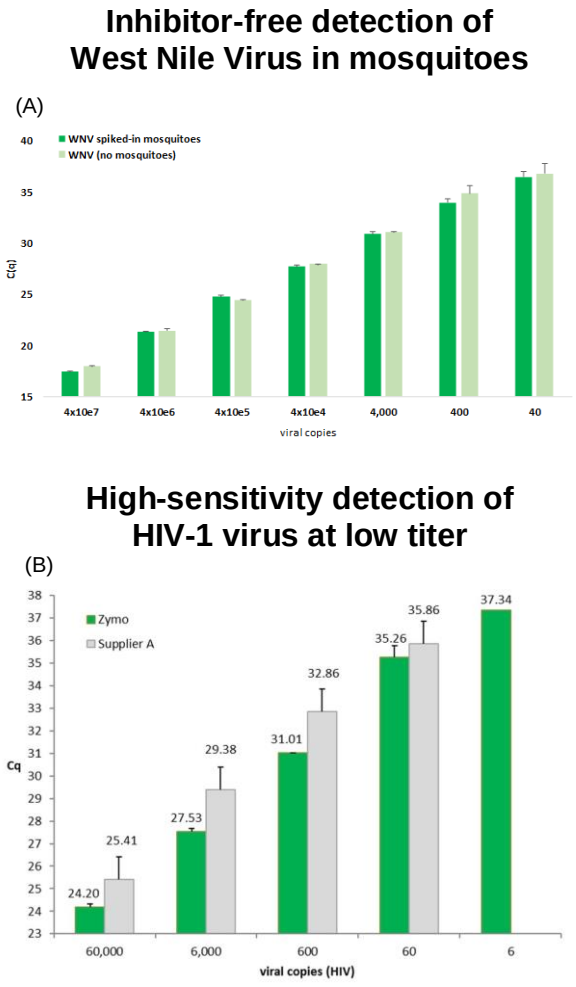
Product Description

***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 **Technical Assistance**, please contact **Zymo** 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)

To download the Automation Script for MagMAX Express 24 –AM1836 protocol, click on the link above or see the product page for R2145, R2146 - Technical Information & Protocol on the Zymo Research website.

Automation Scripts:

***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.

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 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** to reconstitute per 5 mg or 20 mg lyophilized **Proteinase K**, respectively. Vortex to dissolve and store frozen aliquots.

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	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 Appendix, page 5 for specific number of insect input.

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

[3] Catalog no. S6014-50

[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

4. Add 2 µl **Proteinase K** to each 200 µl cleared sample[5] and mix well[6].
5. Add 400 µl **Pathogen DNA/RNA Buffer**[7] and mix well[6].
6. Add 20 µl **ZymoBIOMICS MagBinding Beads** and mix well[8] for 10 minutes.

   Important: ZymoBIOMICS 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[8] and discard the cleared supernatant.
8. Add 500 µl **MagBead DNA/RNA Wash Buffer 1** and mix well[6].
9. Transfer the plate to a magnetic stand until beads have pelleted, then aspirate[8] and discard the cleared supernatant.
10. Add 500 µl **MagBead DNA/RNA Wash Buffer 2** and mix well[6].
11. Transfer the plate to a magnetic stand until beads have pelleted, then aspirate[8] and discard the cleared supernatant.
12. Add 500 µl ethanol (95-100%) and mix well[6].
13. Transfer the plate to a magnetic stand until beads have pelleted, then aspirate[8] 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[9].
16. To elute DNA/RNA from the beads, add 50 µl ZymoBIOMICS **DNase/RNase-Free Water** and mix well[6].

    Alternatively, for highly concentrated DNA/RNA use ≥30 µl volume.
17. Transfer the plate to a magnetic stand until beads have pelleted, then aspirate[8] and dispense the eluted DNA/RNA to an elution plate.

    The eluted DNA/RNA[10] can be used immediately or stored frozen.

Notes:

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

[6] 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.

[7] To ensure efficient lysis and deproteinization, up to 5 volumes of Pathogen DNA/RNA Buffer can be used per 200 µl liquid sample.

[8] 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.

[9] 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).

[10] 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.
***Quick*-DNA/RNA™ Pathogen MagBead**	1x 96 preps 4x 96 preps	R2145 R2146
***Quick*-DNA/RNA™ Pathogen MiniPrep**	50 preps 200 preps	R1042 R1043

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
ZymoBIOMICS MagBinding Beads	3 ml 12 ml	D4302-6-3 D4302-6-12
MagBead DNA/RNA Wash 1 (concentrate)	30 ml 120 ml	R2130-1-30 R2130-1-120
MagBead DNA/RNA Wash 2 (concentrate)	20 ml 80 ml	R2130-2-20 R2130-2-80
ZymoBIOMICS DNase/RNase-Free Water	10 ml 30 ml 50 ml	D4302-5-10 D4302-5-30 D4302-5-50
ZR BashingBead™ Lysis Tubes (0.1 mm & 2.0 mm)	50 pack	S6014-50
ZR-96 MagStand	1	P1005
Collection Plate (capacity 1.2 ml/well)	2 plates	C2002
96-Well Block (capacity 2 ml/well)	2 plates 10 plates	P1001-2 P1001-10
Elution Plate (capacity 0.35 ml/well)	2 plates	C2003
96-Well Plate Cover Foil	2 foils 6 foils 8 foils	C2007-2 C2007-6 C2007-8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The Beauty of Science is to Make Things Simple