ZYMO RESEARCH

RNA Purification Made Simple™

# *Quick*-DNA/RNA™ Pathogen MagBead

DNA & RNA from any vector-borne pathogen

## Highlights

- High-throughput, magnetic-bead 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.).
- 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:
R2145, R2146

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

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

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# Product Contents

| *Quick*-DNA/RNA™ Pathogen MagBead | R2145 (96 prep) | R2146 (4 x 96 prep) |
|---|---|---|
| DNA/RNA Shield™ | 50 ml (x2) | 250 ml (x2) |
| Pathogen DNA/RNA Buffer[1] | 100 ml | 400 ml |
| Proteinase K[2] (lyophilized) | 20 mg | 60 mg |
| Proteinase K Storage Buffer | 1.2 ml (x3) | 10 ml |
| ZymoBIOMICS™ MagBinding Beads | 6 ml | 12 ml (x2) |
| MagBead DNA/RNA Wash 1[3] (concentrate) | 30 ml (x2) | 120 ml (x2) |
| MagBead DNA/RNA Wash 2[4] (concentrate) | 20 ml (x2) | 80 ml (x2) |
| ZymoBIOMICS™ DNase/RNase-Free Water | 30 ml | 50 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 500 µl per 100 ml **Pathogen DNA/RNA Buffer.**
2 Reconstitute lyophilized **Proteinase K** according to page 4, Buffer Preparation. 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 –** ≤ 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 –** 10 µg DNA/RNA per 20 µl **ZymoBIOMICS™ MagBinding Beads**.

- **Elution Volume –** ≥ 50 µl **ZymoBIOMICS™ DNase/RNase-Free Water**.

- **Equipment Needed** (user provided) **–** Beta-mercaptoethanol (b-Me), Isopropanol (99.5-100%), Ethanol (95-100%), Vortex mixer, Magnetic stand or separator, liquid handler or robotic sample processor.

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

  ZR BashingBead™ Lysis Tubes (S6014-50; 0.1 & 2.0 mm beads)
  DNA/RNA Shield™ (R1200; 2X concentrate)
  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 (C2007-4; 4 foils)
  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)

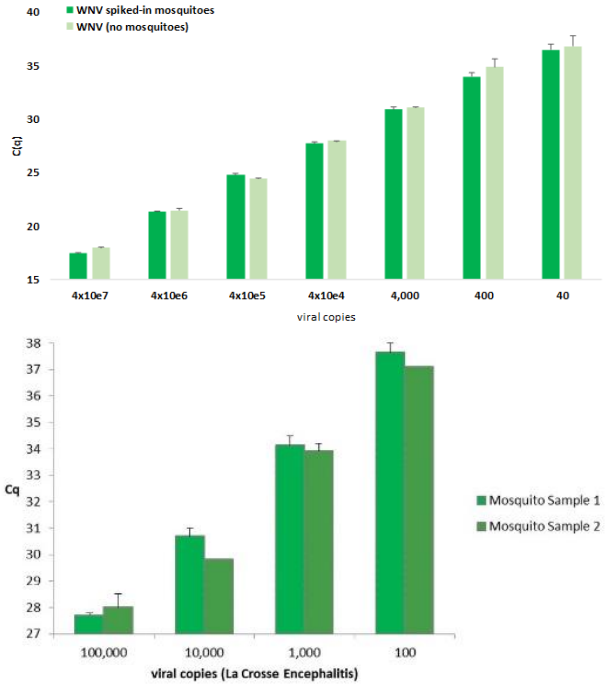
# Product Description

The ***Quick*-DNA/RNA™ Pathogen 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™**. 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 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/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 500 µl beta-mercaptoethanol (user supplied) per 100 ml **Pathogen DNA/RNA Buffer**, (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.
- ✓ Reconstitute lyophilized **Proteinase K** at 20 mg/ml with **Proteinase K Storage Buffer** and mix by vortexing. Use immediately or store frozen aliquots:
  #R2145 (**20 mg**), add 1.04 ml **buffer**
  #R2146 (**60 mg**), add 3.12 ml **buffer**

## (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 and transfer the cleared supernatant into a nuclease-free plate/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<br>5 flat adults<br>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[4].

**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[5] directly to a liquid sample. Mix well[4] 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.).
4 For all buffer additions and incubation steps, **mix well** by pipetting the beads up and down several times and/or by shaking (vortexing) at ~1,300 rpm. Optimization may be required.
5 For automation platforms with "dead volume" liquid handler dispensing, the lyophilized Proteinase K can be reconstituted to a working concentration of 4-20 mg/ml.

## (III) DNA/RNA Purification

- ✓ Perform all steps at room temperature (20-30°C).
- ✓ The sample input can be scaled up or down, proportionally.

1. Add 800 µl **Pathogen DNA/RNA Buffer** to each 400 µl sample[1] (2:1) and mix well[2].
2. Add 20 µl **ZymoBIOMICS™ MagBinding Beads** and mix well[2] for 10 minutes.

   Important: **ZymoBIOMICS™ MagBinding Beads** settle quickly, ensure that beads are kept in suspension while dispensing.
3. Transfer the plate/tube to a magnetic stand[3] until beads have pelleted, then aspirate[4] and discard the cleared supernatant.
4. Add 500 µl **MagBead DNA/RNA Wash 1** and mix well[2]. Pellet the beads[3,4] and discard the supernatant.
5. Add 500 µl **MagBead DNA/RNA Wash 2** and mix well[2]. Pellet the beads[3,4] and discard the supernatant.
6. Add 500 µl ethanol (95-100%) and mix well[2]. Pellet the beads[3,4] and discard the supernatant.
7. Add 500 µl of ethanol (95-100%) and mix well[2]. Then transfer the sample (beads and liquid) to a new plate/tube (not provided). Pellet the beads[3,4] and discard the supernatant.

   Optional: At this point, DNase I treatment can be performed (see Appendices, page 8).
8. Dry the beads for 10 minutes or until fully dry[5].
9. To elute DNA/RNA from the beads, add 50 µl **ZymoBIOMICS™ DNase/RNase-Free Water** and mix well[2].

   Alternatively, for highly concentrated DNA/RNA use ≥ 30 µl volume.
10. Transfer the plate/tube to a magnetic stand[3] until beads have pelleted, then aspirate[4] and dispense the eluted DNA/RNA to a new plate/tube.

    The eluted DNA/RNA[6] can be used immediately or stored frozen.

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1 Up to 400 µl sample can be processed per prep.
2 For all buffer additions and incubation steps, **mix well** by pipetting the beads up and down several times and/or by shaking (vortexing) at ~1,300 rpm. Optimization may be required.
3 Use a strong-field magnetic stand or separator (e.g., ZR-96 MagStand, P1005; sold separately) until beads have pelleted.
4 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.
5 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 (25-55ºC).
6 It is recommended to titrate the DNA/RNA eluate for downstream applications (i.e., RT/qPCR, etc.).

# Appendices

## Automation Scripts

The ***Quick*-DNA/RNA™ Pathogen MagBead** (R2145/R2146) is compatible with automated platforms*. 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.

## DNase I Treatment

✓ For DNA-free RNA, DNase I treatment can be performed using DNase I Set (E1010; 50 reactions) and DNA/RNA Prep Buffer (D7010-2-50), materials sold separately.

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** | |
|---|---|
| Nuclease-free water (not provided) | 40 µl |
| **DNA Digestion Buffer** | 5 µl |
| **DNase I** (reconstituted; 1 U/ul)[1] | 5 µl |

1. Add 50 µl **DNase I Reaction Mix** to the beads and mix gently for 10 minutes.
2. Add 500 µl **DNA/RNA Prep Buffer** and mix well[2] for 10 minutes. Pellet the beads[3,4] and discard the supernatant.
3. Add 500 µl ethanol (95-100%) and mix well[2]. Pellet the beads[3,4] and discard the supernatant.
4. Add 500 µl of ethanol (95-100%) and mix well[2]. Then transfer the sample (beads and liquid) to a new plate/tube (not provided). Pellet the beads[3,4] and discard the supernatant.
5. Proceed with step 8 in DNA/RNA Purification, page 7.

*For automation platforms with "dead volume" liquid handler dispensing, additional reagents are available separately.

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 For all buffer additions and incubation steps, **mix well** by pipetting the beads up and down several times and/or by shaking (vortexing) at ~1,300 rpm. Optimization may be required.

3 Use a strong-field magnetic stand or separator (e.g., ZR-96 MagStand, P1005; sold separately) until beads have pelleted.

4 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.

# Ordering Information

| Product Description | Catalog No. | Size |
|---|---|---|
| ***Quick*-DNA/RNA™ Pathogen MagBead** | R2145<br>R2146 | 96 preps.<br>4 x 96 preps. |

| Individual Kit Components | Catalog No. | Amount |
|---|---|---|
| **Pathogen DNA/RNA Buffer** | R1042-1-50<br>R1042-1-100 | 50 ml<br>100 ml |
| **Proteinase K Set** supplied w/ Storage Buffer | D3001-2-5<br>D3001-2-20 | 5 mg<br>20 mg |
| **MagBead DNA/RNA Wash 1** (concentrate) | R2130-1-30<br>R2130-1-120 | 30 ml<br>120 ml |
| **MagBead DNA/RNA Wash 2** (concentrate) | R2130-2-20<br>R2130-2-80 | 20 ml<br>80 ml |
| **ZymoBIOMICS™ MagBinding Beads** | D4302-6-6<br>D4302-6-12 | 6 ml<br>12 ml |
| **ZymoBIOMICS™ DNase/RNase-Free Water** | D4302-5-30<br>D4302-5-50 | 30 ml<br>50 ml |
| **DNA/RNA Shield™** | R1100-50<br>R1100-250 | 50 ml<br>250 ml |
| **DNA/RNA Shield™ Fecal Collection Tube** | R1101 | 10 |
| **DNA/RNA Shield™ Collection Tube**<br>**DNA/RNA Shield™ Lysis Tube** (microbe)<br>**DNA/RNA Shield™ Lysis Tube** (microbe) w/ swab<br>**DNA/RNA Shield™ Lysis Tube** (tissue) | R1102<br>R1103<br>R1104<br>R1105 | 50<br>50<br>50<br>50 |
| **DNA/RNA Shield™ Collection Tube w/ Swab** (1 ml fill) | R1106<br>R1107 | 10<br>50 |
| **DNA/RNA Shield™ Collection Tube w/ Swab** (2 ml fill) | R1108<br>R1109 | 10<br>50 |
| **DNA/RNA Shield™ Saliva Collection Kit** (2 ml fill) | R1210 | 1 |
| **ZR-96 MagStand** | P1005 | 1 |
| **Collection Plate** (capacity 1.2 ml/well) | C2002 | 2 |
| **96-Well Block** (capacity 2 ml/well) | P1001-2 | 2 |
| **Elution Plate** (capacity 0.35 ml/well) | C2003 | 2 |
| **96-Well Plate Cover Foil** | C2007-2<br>C2007-4 | 2<br>4 |
| **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<br>DNA/RNA Shield™ Lysis Tube (microbe)<br>DNA/RNA Shield™ Lysis Tube (microbe) w/ swab<br>DNA/RNA Shield™ Lysis Tube (tissue)<br>#R1102-R1105 | For microbes, tissue, etc.<br>(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** | |
|---|---|
| MagBeads #R1081, R1082 | DNase I Set included (#R1082) |

# Troubleshooting Guide

| Problem | Possible Causes and Suggested Solutions |
|---|---|
| **RNA degradation** | To prevent RNA degradation:<br><br>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 (blood, plasma/serum, tissue etc.):<br><br>- Increase the volume of DNA/RNA Shield™ added to the sample.<br><br>- Perform Proteinase K treatment (see Sample Preparation, page 5).<br><br>Increase binding time:<br><br>At all binding steps, increase binding time for an additional ≥ 10 minutes (e.g., 30 minutes). Depending on the amount of biomass, more time may be required to allow nucleic acids to be sufficiently bound to beads.<br><br>Inefficient washing of beads:<br><br>-Shaking/Mixing: Mix well by pipetting up and down several times and/or by shaking (vortexing) at high speed. Make sure that the beads are resuspended throughout the bind, wash and elution steps.<br><br>Increase eluate input:<br><br>-Titrate the DNA/RNA eluate for downstream applications (i.e., RT/qPCR). |
| **DNA contamination** | To remove DNA:<br><br>- Perform DNase I treatment during the purification (page 8) or perform DNase I treatment post-purification (#R1082), then clean-up the treated sample. |

For technical assistance, please contact 1-888-882-9682 or email tech@zymoresearch.com

# Notes


**100% satisfaction guarantee on all Zymo Research products, or your money back.**

Zymo Research is committed to simplifying your research with quality products and services. If you are dissatisfied with this product for any reason, please call 1(888) 882-9682.

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.

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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