ZYMO RESEARCH

RNA Purification Made Simple™

Quick-DNA/RNA™ Viral MagBead

Viral DNA & RNA from any biological sample

Highlights

- High-throughput, magnetic-bead based purification of viral DNA and RNA from plasma, serum, urine, cell culture media, blood, saliva, cellular suspensions, swab, fecal and biopsy samples
- 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:
R2140, R2141

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

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Product Contents

Quick-DNA/RNA™ Viral MagBead	R2140 (250 preps)	R2141 (1000 preps)
DNA/RNA Shield™ (2X concentrate)	25 ml	125 ml
Viral 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
MagBinding Beads	3 ml	12 ml
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)
DNase/RNase-Free Water	30 ml	100 ml
Instruction Manual	1 pc	1 pc

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature (15-30°C).
Before use:
1 Add beta-mercaptoethanol to 0.5% (v/v) *i.e.,* add 500 µl per 100 ml **Viral DNA/RNA Buffer.**
2 Reconstitute lyophilized **Proteinase K** according to page 4, Buffer Preparation. Store frozen aliquots.
3 Add 20 ml (R2140) or 80 ml (R2141) of isopropanol to the **MagBead DNA/RNA Wash 1** concentrate.
4 Add 30 ml (R2140) or 120 ml (R2141) of isopropanol to the **MagBead DNA/RNA Wash 2** concentrate.

Specifications

- **Sample Sources –** ≤ 200 µl plasma, serum, saliva, swab, urine, cell culture media, blood, cellular suspension, fecal sample or ≤ 5 mg biopsy sample.

  For samples in UTM®/VTM®, PBS or saline, see Sample Preparation, page 5.

- **Purity –** DNA/RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.

- **Binding Capacity –** 5 µg DNA/RNA per 10 µl **MagBinding Beads**.

- **Elution Volume –** ≥ 15 µl **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-96 MagStand (P1005)
  Collection Plate (C2002; capacity 1.2 ml/well)
  96-Well Block (P1001-2; 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)

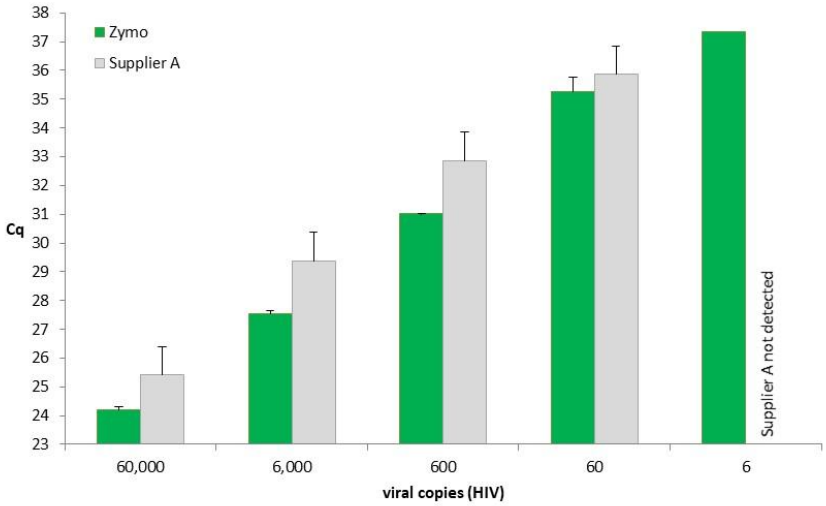
Product Description

The ***Quick*-DNA/RNA™ Viral MagBead** kit is designed for high-throughput purification of viral DNA and/or RNA from plasma, serum, urine, cell culture media, blood, saliva, cellular suspensions, biopsies, swab and fecal samples stored in **DNA/RNA Shield**™ (for sample collection, nucleic acid preservation and inactivation of pathogens).

The kit also features a buffer system that facilitates complete viral particle lysis for efficient nucleic acid isolation. Small (> 50 nt) and large (> 200 kb) DNA and RNA are bound to magnetic beads, washed and eluted.

The isolated high-quality nucleic acids are ready for all downstream applications such as Next-Gen sequencing, hybridization-based and RT/qPCR detection.

High-Sensitivity Viral Detection at Low Titer



Viral RNA isolated from plasma samples using the ***Quick*-DNA/DNA™ Viral MagBead** kit.
Image shows average Cq values (in duplicate; +/- SD) from RT-qPCR in comparison to Supplier A.

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 **Viral DNA/RNA Buffer**, (final 0.5% (v/v)).
- ✓ Add 20 ml (R2140) or 80 ml (R2141) of isopropanol to the **MagBead DNA/RNA Wash 1** concentrate.
- ✓ Add 30 ml (R2140) or 120 ml (R2141) of isopropanol to the **MagBead DNA/RNA Wash 2** concentrate.
- ✓ To prepare DNA/RNA Shield™ (1X), dilute the **2X concentrate** with an equal volume of nuclease-free water (not provided).
- ✓ Reconstitute lyophilized **Proteinase K** at 20 mg/ml with **Proteinase K Storage Buffer** and mix by vortexing. Use immediately or store frozen aliquots:
  #R2140 (**20 mg**), add 1.04 ml **buffer**
  #R2141 (**60 mg**), add 3.12 ml **buffer**

(II) Sample Preparation

- ✓ Perform all steps at room temperature (15-30°C).
- ✓ Depending on sample type, up to 200 µl can be processed per prep (see below).

Samples in DNA/RNA Shield™[1,2] collection devices (swabs, saliva, etc.)
Transfer up to 200 µl and proceed directly with purification, page 6.

Swabs (UTM®/VTM®, PBS, saline, etc.)
Transfer up to 200 µl and proceed directly with purification, page 6.
Optional - To inactivate, store and preserve samples at room temperature prior to further processing, add **DNA/RNA Shield™**. See **Liquids**, below.

Liquids (plasma[2], serum[2], CSF, blood, saliva, urine, cell suspension, cell culture media)
Add 100 µl of **DNA/RNA Shield™** (2X concentrate) to 100 µl liquid sample (1:1) and mix well[3]. Transfer up to 200 µl of the mixture and proceed with purification, page 6.

Tissue[2] (LCM, needle biopsy)
Add 200 µl **DNA/RNA Shield™** (1X) to a tissue sample (up to 5 mg) and mix well[3]. Proceed with purification, page 6.

Optional - **Proteinase K treatment** (protein-rich samples e.g., plasma, serum, saliva, sputum, tissue, can be treated):

Add 1% **Proteinase K** (v/v) at 20 mg/ml[4] directly to a liquid sample. Mix well[3] and incubate at room temperature for 15 minutes. Note: Up to 5% Proteinase K can be added (e.g., tissue). For example: Add 2-10 µl Proteinase K to each 200 µl sample.

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1 At this point, samples in DNA/RNA Shield™ can be stored at ambient temperature (4-30°C) for a month, 7 days at 37°C, or long-term (> 1 year) -20°C or below.
2 To remove particulate debris or cryoprecipitates (if any), centrifuge and transfer up to 200 µl of the cleared supernatant into a nuclease-free plate/tube (not provided).
3 For all buffer additions and incubation steps, **mix well** for ≥1 minute, by pipetting the beads up and down and/or by shaking (vortexing) at ~1,300 rpm. Optimization may be required.
4 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 (15-30°C).

1. Add 400 µl **Viral DNA/RNA Buffer** to each 200 µl sample[1] (2:1) and mix well[2].
2. Add 10 µl **MagBinding Beads** and mix well[2] for 10 minutes.

   Important: **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 250 µl **MagBead DNA/RNA Wash 1** and mix well[2]. Pellet the beads[3,4] and discard the supernatant.
5. Add 250 µl **MagBead DNA/RNA Wash 2** and mix well[2]. Pellet the beads[3,4] and discard the supernatant.
6. Add 250 µl ethanol (95-100%) and mix well[2]. Pellet the beads[3,4] and discard the supernatant.
7. Add 250 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 7).
8. Dry the beads for 10 minutes or until fully dry[5].
9. To elute DNA/RNA from the beads, add 30 µl **DNase/RNase-Free Water** and mix well[2].

   Alternatively, for highly concentrated DNA/RNA use ≥ 15 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 200 µl sample (including the volume of DNA/RNA Shield, if added) can be processed per prep.
2 For all buffer additions and incubation steps, **mix well** for ≥1 minute, by pipetting the beads up and down 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™ Viral MagBead** (R2140/R2141) 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 250 µl ethanol (95-100%) and mix well[2]. Pellet the beads[3,4] and discard the supernatant.
4. Add 250 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 6.

*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** for ≥1 minute, by pipetting the beads up and down 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™ Viral MagBead**	R2140 R2141	250 preps. 1000 preps.

Individual Kit Components	Catalog No.	Amount
Viral DNA/RNA Buffer	D7020-1-25 D7020-1-100	25 ml 100 ml
Proteinase K Set supplied w/ Storage Buffer	D3001-2-5 D3001-2-20	5 mg 20 mg
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
MagBinding Beads	D4100-2-3 D4100-2-12	3 ml 12 ml
DNase/RNase-Free Water	W1001-30 W1001-100	30 ml 100 ml
DNA/RNA Shield™ (2X concentrate)	R1200-25 R1200-125	25 ml 125 ml
DNA/RNA Shield™ Fecal Collection Tube	R1101	10
DNA/RNA Shield™ Collection Tube **DNA/RNA Shield™ Lysis Tube** (microbe) **DNA/RNA Shield™ Lysis Tube** (microbe) w/ swab **DNA/RNA Shield™ Lysis Tube** (tissue)	R1102 R1103 R1104 R1105	50 50 50 50
DNA/RNA Shield™ Collection Tube w/ Swab (1 ml fill)	R1106 R1107	10 50
DNA/RNA Shield™ Collection Tube w/ Swab (2 ml fill)	R1108 R1109	10 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 C2007-4	2 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 DNA/RNA Shield™ Lysis Tube (microbe) DNA/RNA Shield™ Lysis Tube (microbe) w/ swab DNA/RNA Shield™ Lysis Tube (tissue) #R1102-R1105	For microbes, tissue, etc. (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: 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.): - Increase the volume of DNA/RNA Shield™ to the sample. - Perform Proteinase K treatment (see Sample Preparation, page 4). Increase binding time: 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. Inefficient washing of beads: -Shaking/Mixing: Mix well for ≥1 minute, by pipetting up and down and/or by shaking (vortexing) at high speed. Make sure that the beads are resuspended throughout the bind, wash and elution steps. Optimization may be required. Increase eluate input: -Titrate the DNA/RNA eluate for downstream applications (i.e., RT/qPCR).
DNA contamination	To remove DNA: - Perform DNase I treatment during the purification (page 6) 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

Notes

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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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