ZYMO RESEARCH

Quick-DNA/RNA™ Water Kit

Isolation of Inhibitor-free Nucleic Acid from Water Samples

Highlights

- Concentrate and purify total DNA and/or RNA from small and large volume water samples.
- Includes a sample stabilization reagent that captures viruses, microbes and free nucleic acids without filtration or ultracentrifugation.
- Purified DNA and/or RNA is inhibitor-free and ready for any downstream application including NGS and PCR (i.e. dPCR, RT-PCR, etc.).

Catalog Number:
R2044

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Table of Contents

Product Contents

Quick-DNA/RNA™ Water Kit	R2044 (50 Preps.)
DNA/RNA Shield™	50 ml
Wastewater Stabilization Buffer	250 ml
Viral DNA/RNA Buffer[1]	2 x 25 ml
DNA/RNA Binding Buffer	50 ml
DNA/RNA Prep Buffer	50 ml
DNA/RNA Wash Buffer[2] (concentrate)	24 ml
DNase/RNase-Free Water	10 ml
DNase I[3] (lyophilized)	250 U
DNA Digestion Buffer	4 ml
Zymo-Spin™ IIICG Columns	2 x 50
Zymo-Spin™ IV-IR HRC Filters	50
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	50
Collection Tubes	250
Instruction Manual	1 pc

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 125 µl β-Me per 25 ml **Viral DNA/RNA Buffer.**
[2] Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate.
[3] Reconstitute lyophilized **DNase I (E1009-1 (250 U))** with 275 µl **DNase/RNase-Free Water,** mix by gentle inversion, and store as frozen aliquots.

Specifications

- **Sample Sources –** Water, wastewater, sewage, sludge, finished water, natural water, river water, fresh water, salt water, etc.

- **Sample Size –** Up to 1 L low biomass liquid samples
  Up to 50 ml raw wastewater samples

  Note: A larger sample volume can be used with the appropriate centrifugal rotor and compatible centrifuge bottle.

- **DNA/RNA Purity –** High quality, inhibitor-free DNA/RNA suitable for all downstream applications including NGS, qPCR, dPCR, RT-qPCR, and RT-dPCR.

- **Yield –** Up to 25 µg DNA and/or 100 µg RNA can be eluted into ≥ 50 µl allowing for a highly concentrated sample.

- **DNA/RNA Storage –** DNA and/or RNA is eluted with DNase/RNase-Free Water and can be stored at ≤ -70°C. The addition of RNase inhibitors is highly recommended for prolonged storage.

- **Equipment Needed** (user provided) **–** Microcentrifuge, vortex, and floor model centrifuge capable of spinning 50 ml conical tubes.

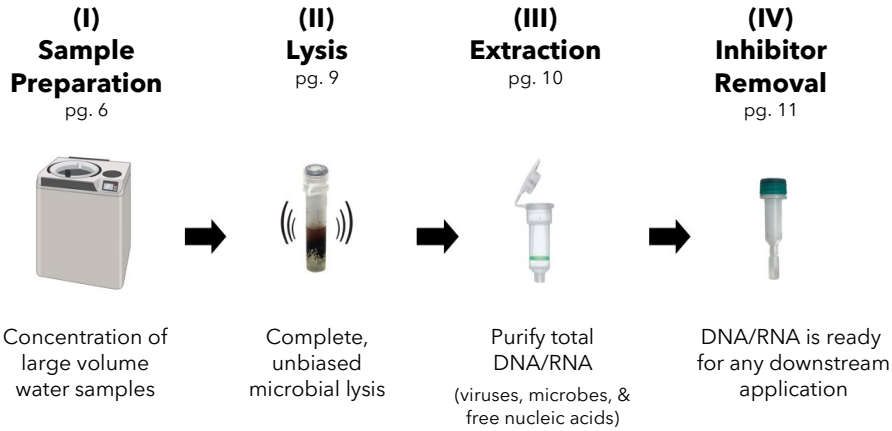
Product Description

The ***Quick*-DNA/RNA™ Water Kit** provides inhibitor-free nucleic acid isolation from up to 50 ml of raw wastewater or higher volumes of low-biomass water samples. The kit includes **Wastewater Stabilization Buffer**, a specialized solution for wastewater sample preparation, and a novel inhibitor removal technology to ensure eluted DNA/RNA is ready for any downstream application.

Wastewater Stabilization Buffer facilitates concentration of viruses, microbes, and free nucleic acids eliminating the need for vacuum filtration. This buffer also enables pathogen inactivation when added to water samples and stabilizes DNA/RNA for up to 1 week at ambient temperatures allowing for safe, cold chain-free storage and transportation.

The DNA/RNA purification workflow includes the novel **Zymo-Spin™ IV-IR HRC** inhibitor removal technology for robust nucleic acid isolation.

The *Quick*-DNA/RNA™ Water Kit Ensures High Recovery of Inhibitor-free Nucleic Acid from Wastewater



Superior Pathogen Detection from Wastewater

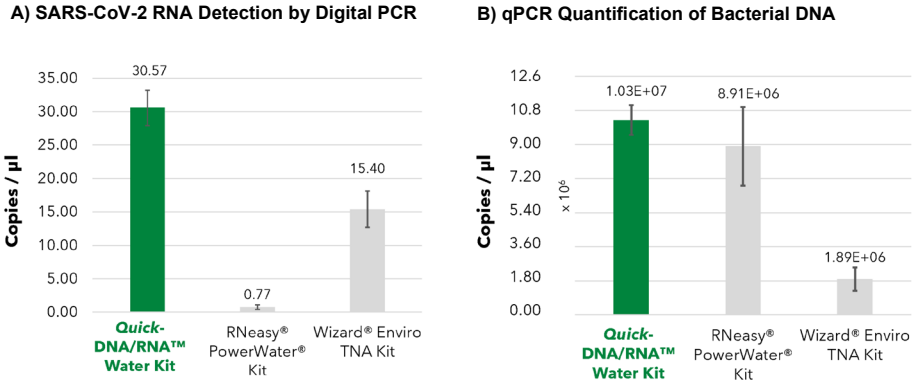


Figure 1. *Quick*-DNA/RNA™ Water Kit provides enhanced detection of pathogens. Nucleic acids were purified from 7.5 ml of influent wastewater containing SARS-CoV-2 virus using the *Quick*-DNA/RNA™ Water Kit and other commercial kits (n=3). **A)** Digital PCR was used to detect SARS-CoV-2 (N2 gene) in eluates from each kit, with viral RNA quantified using the Bio-Rad™ PREvalence ddPCR™ SARS-CoV-2 Wastewater Quantification Kit. **B)** Bacterial DNA recovery was assessed and quantified by qPCR using the Femto Bacterial DNA Quantification Kit.[1]

Sensitive and Linear Recovery of Nucleic Acids

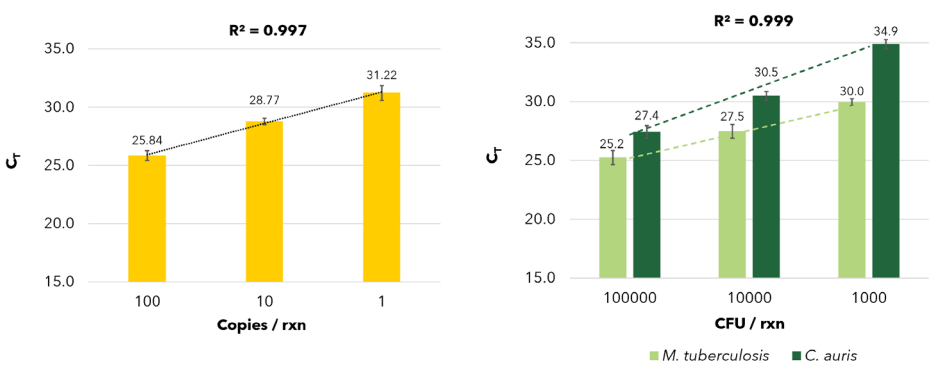


Figure 2. The *Quick*-DNA/RNA™ Water Kit demonstrates linear recovery for virus, bacterial, and fungal targets. Heat-inactivated SARS-CoV-2 virus, *Candida auris*, and *Mycobacterium tuberculosis* were spiked into 10 mL of influent wastewater aliquots at varying concentrations, with all targets absent in the native wastewater sample. Nucleic acids were then extracted and purified using the *Quick*-DNA/RNA™ Water Kit. **A)** Viral RNA recovery was quantified by RT-qPCR using the Quick SARS-CoV-2 Multiplex Kit[2], shown as genome equivalent copies per PCR reaction. **B)** *C. auris* (fungi) and *M. tuberculosis* (bacteria) were measured by qPCR with target-specific primers, with results displayed as colony-forming units (CFU) per PCR reaction.

[1] **Femto Bacterial DNA Quantification Kit** (E2006) is sold separately.
[2] ***Quick* SARS-CoV-2 Multiplex Kit** (R3013) is sold separately.

Protocol

The protocol covers: (I) Sample Preparation, (II) Sample Lysis, (III) DNA/RNA Purification, and (IV) Inhibitor Removal steps.

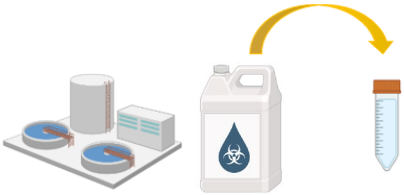
Buffer Preparation

- ✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate.
- ✓ Add beta-mercaptoethanol (user provided) to 0.5% (v/v) i.e. add 125 µl β-Me per 25 ml **Viral DNA/RNA Buffer**.

(I) Sample Preparation

- ✓ Perform all steps at room temperature (15-30°C).
- ✓ For viral enrichment, see appendix.

Liquid Samples (raw wastewater, sewage, natural and finished water)



Liquid sample from collection site

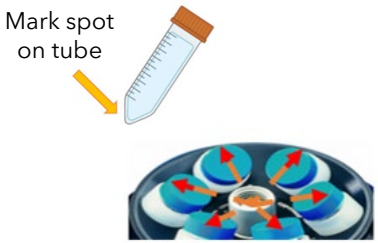
1. Transfer up to 45 ml of the collected liquid sample into 50 ml conical tube compatible with floor model centrifuge.

Note: A larger sample volume can be used with the appropriate centrifugal rotor and compatible centrifuge bottle.

≤50 mL liquid sample

2. Add 0.1 volume of **Wastewater Stabilization Buffer** to the liquid sample. Mix well by vortexing. Incubate at room temperature for 10 minutes.[1]

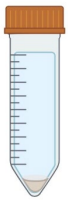
Example: Add 4.5 ml of Wastewater Stabilization Buffer to 45 ml liquid sample.



Place tube with mark facing outward on the rotor.

3. Centrifuge at 10,000 *x g* for 20 minutes. Mark a location near the bottom of the tube and orient outward on the rotor during centrifugation. This will aid in the resuspension of pellets that may not be clearly visible.

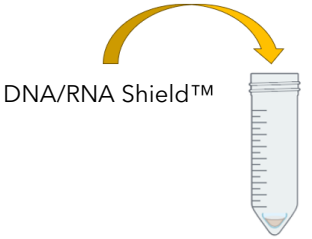
[1]After incubation with **Wastewater Stabilization Buffer**, liquid samples can be stored at ambient temperature for up to 1 week or frozen at ≤ -70°C for long term storage.



Slowly and carefully decant.

4. Without disturbing the pellet, slowly decant or remove the supernatant leaving behind ~250 µl of liquid. Discard the supernatant.

   Note: Pellet may not be visible. Carefully avoid fast supernatant removal over the marked location.



5. Resuspend the pellet with 750 µl **DNA/RNA Shield™** (1X concentrate). Pipette thoroughly to mix. Proceed to **(II) Sample Lysis** on pg. 9.

   Optional Stopping Point: *Samples can be stored for several hours at room temperature or ≤ -70°C for long-term storage.*

Samples in Wastewater Sample Collection Bottles[1]

1. Transfer up to 50 ml of sample into conical tube compatible with floor model centrifuge. A larger sample volume can be used with the appropriate centrifugal rotor and compatible centrifuge bottle.
2. Continue from Step 3 of the **Liquid Samples** workflow on page 6.

Water Filters

1. Cut the filter into small pieces and place into **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)**.
2. Add 750 µl **DNA/RNA Shield™** (1X concentrate) to the tube and cap tightly.
3. Proceed to Step 2 of **(II) Sample Lysis** on page 9.

[1] **Wastewater Sample Collection Bottles** (R1503, R1503-10) are sold separately.

Wastewater Solids and Sludge

1. Transfer up to 250 mg of sample directly into **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)**.
2. Add 750 µl **DNA/RNA Shield™** (1X concentrate) to the tube and cap tightly.
3. Proceed to Step 2 of **(II) Sample Lysis** on page 9.

Concentrated Water Samples

Molecular Cut-Off Filters
Please follow the instructions provided by the filter manufacturer for processing water samples.
After processing the sample with molecular cut-off filter, transfer the concentrated water sample to a new DNase/RNase-Free tube and increase volume to 1 ml with **DNA/RNA Shield™** (1X concentrate). Proceed with **(II) Sample Lysis** on page 9.

Ceres Nanosciences Nanotrap® Particles
Please follow the instructions provided by Ceres Nanosciences. After processing with Nanotrap Enhancement Reagents, Nanotrap® Particles should be separated from the sample (containing concentrated microbes/viruses). Transfer the sample to a new DNase/RNase-free tube and increase volume to 1 ml with **DNA/RNA Shield™** (1X concentrate). Proceed to **(II) Sample Lysis** on page 9.

(II) Sample Lysis (recommended for complete microbial lysis)

- ✓ Perform all steps at room temperature (15-30°C), unless specified.
- ✓ For viral enrichment, see appendix.

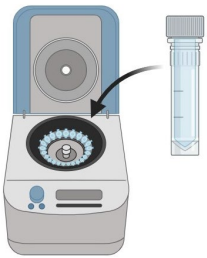
Transfer sample to ZR BashingBead™ Lysis Tube

1. Transfer up to 1 ml of the sample to **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)**, and cap tightly.

Homogenize

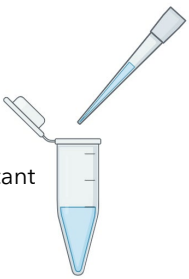
2. Secure prepared lysis tube in bead beater fitted with 2 ml tube holder assembly and process using optimized beat beating conditions (speed and time) for your device (see Appendix).

   Optional Stopping Point: *Samples can be stored for several hours at room temperature or ≤ -70°C for long term storage.*



Centrifuge lysis tube.

3. Centrifuge lysis tube at 16,000 *x g* for 1 minute.



Transfer supernatant to new tube.

4. Transfer up to 400 µl of supernatant to new DNase/RNase-Free tube.

5. Proceed to **(III) DNA/RNA Purification** on page 10.

(III) DNA/RNA Purification

- ✓ Perform all steps at room temperature (15-30°C) and centrifugation at 16,000 *x g* for 1 minute, unless specified.
- ✓ For all buffer additions, mix well by pipetting up and down and/or by vortexing for 1-2 seconds, unless specified.

1. Add 800 µl of **Viral DNA/RNA Buffer** to the supernatant and mix well. Transfer the mixture into a **Zymo-Spin™ IIICG Column** in a **Collection Tube,** centrifuge[1] and discard the flow-through.

   Optional: A vacuum manifold may be used instead of centrifuge.

2. Add 400 µl **DNA/RNA Prep Buffer** to the column, centrifuge and discard the flow-through.

3. Add 100 µl **DNase/RNase-Free Water** directly to the column matrix and incubate at room temperature for 5 minutes. Centrifuge and **save this eluted DNA/RNA!**

4. To the eluted DNA/RNA from step 3, add 200 µl of **DNA/RNA Binding Buffer** and mix well.

5. Add 400 µl ethanol (95-100%) to the mixture and mix well.

6. Transfer the entire mixture into a new **Zymo-Spin™ IIICG Column** in a **Collection Tube** and centrifuge. Discard the flow-through.

   Optional: At this point, **DNase I Treatment** can be performed. See Appendix.

7. Add 400 µl **DNA/RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.

8. Add 700 µl **DNA/RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.

9. Transfer the column carefully into a new **Collection Tube** and centrifuge to remove any residual wash buffer. Carefully, transfer column into a nuclease-free tube (not provided).

10. Add 100 µl of **DNase/RNase-Free Water** directly to the column matrix. Incubate at room temperature for 5 minutes, then centrifuge to elute the DNA and/or RNA.

    Alternatively, for highly concentrated DNA and/or RNA use ≥ 50 µl elution.

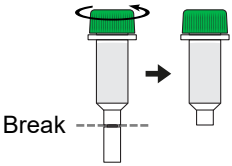
    Optional Stopping Point: *If needed, the eluted DNA and/or RNA can be stored at ≤ -70°C before continuing with* ***(IV) Inhibitor Removal****.*

[1] To process samples > 750 µl, reload the column.

(IV) Inhibitor Removal

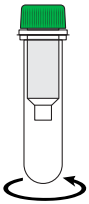
✓ Perform all steps at room temperature (15-30°C), unless specified.

1. Loosen the **Zymo-Spin™ IV-IR HRC Filter** screw cap and break bottom tip off filter.



2. Insert the filter into a collection tube.

3. Centrifuge at 8,000 *x g* for 3 minutes.



4. Transfer the prepared filter to a clean 1.5 ml microcentrifuge tube. Add the eluate (containing DNA and/or RNA) to the **Zymo-Spin™ IV-IR HRC Filter** and centrifuge at 8,000 *x g* for 2 - 3 minutes.[1]

   Filtered DNA and/or RNA is now suitable for PCR, RT, NGS and any other downstream applications.[2]

   The eluted DNA and/or RNA can be used immediately or stored at ≤ -70°C.

[1] Use 3-minute centrifugation for volumes <100 µl.
[2] HRC matrix chemistry may skew the A260/230 purity value. This will **not** affect any downstream processes.

Appendices

Sample Collection

For water samples collected via autosampler or grab devices, it is recommended to add **Wastewater Stabilization Buffer** as soon as possible to best preserve nucleic acids and microbial profiles. The Wastewater Stabilization Buffer inactivates pathogens, allowing for safe handling and reducing potential health risks. It also protects sample integrity at ambient temperature, enabling storage for up to 7 days and removes the need for cold chain shipping.

Wastewater Sample Collection Bottle—A wide mouth HPDE bottle that is pre-filled with Wastewater Stabilization Buffer. Simply fill up the bottle with the liquid sample, mix, and store at ambient temperature for up to 1 week before processing. Safely collect, preserve, and transport water samples. **(Cat No. R1503, R1503-10)**

Viral Enrichment

To enrich for viruses, the following protocol can be performed.

1. Transfer up to 50 ml of collected liquid sample into a conical tube compatible with floor model centrifuge.

   Note: A larger sample volume can be used with the appropriate centrifugal rotor and compatible centrifuge bottle.

2. Centrifuge at 4,000 *x g* for 2 minutes to pellet debris.[1]
3. Carefully decant and transfer supernatant to new conical tube. **Save the supernatant!** This step will partially remove bacteria, fungi, and debris from high turbidity water samples.
4. Continue following Step 2 – 4 of **(I) Sample Preparation.**
5. Resuspend the pellet with 250 µl **DNA/RNA Shield™** (1X concentrate). Pipette thoroughly to mix.
6. Incubate the resuspended sample for 15 minutes at room temperature.
7. Centrifuge at 16,000 *x g* for 1 minute.
8. Transfer up to 400 µl of supernatant to new DNase/RNase-Free tube.
9. Proceed to **(III) DNA/RNA Purification** on page 10.

[1] Centrifugation speed and time should be optimized based on sample turbidity.

DNase I Treatment

✓ For DNA-free RNA, DNase I treatment can be performed.

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	
DNA Digestion Buffer	75 µl
DNase I[1] (reconstituted; 1 U/µl)	5 µl

1. Following Step 6 of **(III) DNA/RNA Purification**, add 400 µl **DNA/RNA Wash Buffer**[2] to the column and centrifuge. Discard the flow-through.
2. Add 80 µl DNase I Reaction Mix directly to the matrix of the column.
3. Incubate at room temperature (15-30°C) for 15 minutes.
4. Proceed with Step 7 of **(III) DNA/RNA Purification** on page 10.

Before use:
[1] Reconstitute lyophilized **DNase I (E1009-1 (250 U))** with 275 µl **DNase/RNase-Free Water,** mix by gentle inversion, and store as frozen aliquots.
[2] Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate (D7010-3-24).

Optimized Lysis Protocols

The following conditions with different mechanical lysis machines were validated with minimum bias using the ZymoBIOMICS® Microbial Community Standard (D6300).

1 Vortex Genie with 2ml BashingBead™ Tubes

Recommended for ease of use and accessibility

Use Microtube Adaptor (Scientific Industries, Inc. Cat. No. S5001-7)

1. 40 minutes of continuous bead beating (max of 18 tubes per adaptor)

2 Bertin Precellys Evolution with 2 ml BashingBead™ Tubes

Recommended for ease of use and ultra-high speed.

1. 1 minute on at 9,000 RPM
2. 2 minutes rest
3. Repeat cycle 4 times for a total of 4 minutes of bead beating

3 MP Fastprep-24™ (Classic & 5G) with 2 ml BashingBead™ Tubes

Maximum of 20 tubes. The weight of > 20 tubes may cause a system error.

1. 1 minute on at 6.5 m/s
2. 5 minutes rest
3. Repeat cycle 5 times for a total of 5 minutes of bead beating

4 Omni Bead Ruptor Elite with 2 ml BashingBead™ Tubes

1. 1 minute on at 6 m/s
2. 5 minutes rest
3. Repeat cycle 3 times for a total of 3 minutes of bead beating

5 Biospec Mini-BeadBeater-16 with 2 ml BashingBead™ Tubes

1. 1 minute at maximum speed
2. 5 minutes rest
3. Repeat cycle 5 times for a total of 5 minutes of bead beating

6 Biospec Mini-BeadBeater-96 with 2 ml BashingBead™ Tubes

1. 5 minutes on at Max RPM
2. 5 minutes rest
3. Repeat cycle 4 times for a total of 20 minutes of bead beating

7 Biospec Mini-BeadBeater-96 with 96 well lysis rack

1. 5 minutes on at Max RPM
2. 5 minutes rest
3. Repeat cycle 8 times for a total of 40 minutes of bead beating

✕ TissueLyser II

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

✕ TissueLyser LT

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

✕ Retsch Mixer Mill MM 400

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

Ordering Information

Amount	Catalog No.	Size
***Quick*-DNA/RNA™ Water Kit**	R2044	50 preps.

Individual Kit Components	Catalog No.	Amount
DNA/RNA Shield™	R1100-50 R1100-250	50 ml 250 ml
Viral DNA/RNA Buffer	D7020-1-25 D7020-1-100	25 ml 100 ml
DNA/RNA Binding Buffer	D7010-1-10 D7010-1-25 D7010-1-50	10 ml 25 ml 50 ml
DNA/RNA Prep Buffer	D7010-2-10 D7010-2-25 D7010-2-50 D7010-2-200	10 ml 25 ml 50 ml 200 ml
DNA/RNA Wash Buffer (concentrate)	D7010-3-6 D7010-3-12 D7010-3-24	6 ml 12 ml 24 ml
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10	1 ml 4 ml 6 ml 10 ml
Zymo-Spin™ IIICG Columns	C1006-50-G C1006-250-G	50 250
Zymo-Spin™ IV-IR HRC Filters	C1010-50	50
DNase I Set	E1010 E1011 E1012	250 U 1500 U 5 x 1500 U
ZR Lysis BashingBead™ Tubes (0.1 & 0.5 mm)	S6012-50	50
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000

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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