SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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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.
- Eluted, inhibitor-free DNA and/or RNA is ready for any downstream application including NGS and PCR (i.e. ddPCR, RT-PCR, etc.).

Catalog Number:
R2044

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

Table of Contents

Product Contents

Quick-DNA/RNA™ Water Kit	R2044 (50 Preps.)
DNA/RNA Shield™	50 ml
Wastewater Stabilization Buffer	140 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
Inhibitor Removal Binding Buffer	30 ml
Inhibitor Removal Wash Buffer	2 x 10 ml
Inhibitor Removal Elution Buffer	5 ml
MAGicBead™ Mix	1.5 ml
Zymo-Spin™ IIICG Columns	2 x 50
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	50
Collection Tubes	200
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 sample

  Note: It is recommended to use 50 mL conical tubes when pelleting from water samples of 45 mL or less. A floor model centrifuge should be used if available.

- **DNA/RNA Purity –** High quality, inhibitor-free DNA/RNA (A_{260}/A_{280} > 1.8, A_{260}/A_{230} > 1.8) suitable for all downstream applications including NGS, qPCR, ddPCR, RT-qPCR, and RT-ddPCR.

- **Yield –** Up to 25 µg DNA 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 Inhibitor Removal Elution Buffer and can be stored at ≤ -70°C. The addition of RNase inhibitors is highly recommended for prolonged storage.

- **Equipment Needed** (user provided) **–** Microcentrifuge, vortex, floor model centrifuge capable of spinning 50 ml conical tubes, magnetic stand[1], and rotator capable of at least 30 rpm.

[1] Recommended magnetic stand: Promega MagneSphere® Magnetic Separation Stand or Permagen® Centrifuge Magnetic Separation Rack

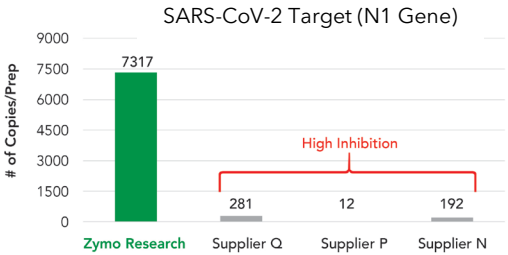
Product Description

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 acid eliminating the need for vacuum filtration. This buffer also facilitates 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.

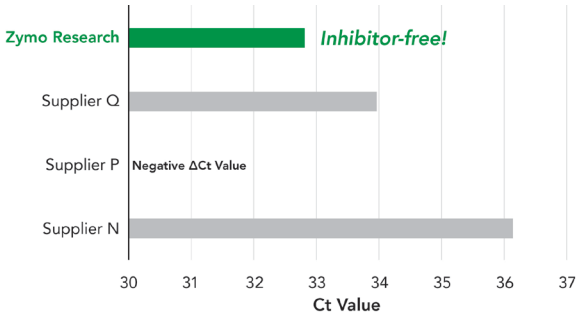
The DNA/RNA purification workflow includes novel **MAGicBead™** binding chemistry for robust, scalable nucleic acid isolation.

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

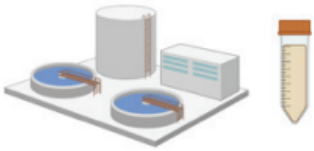


Viral RNA Detection
Figure 1. Comparison of detection of SARS-CoV-2 in raw wastewater by RT-qPCR using *Quick*-DNA/RNA™ Water Kit and other commercial kits.

PCR Inhibition
Figure 2. Comparison of inhibition level in the final eluate using *Quick*-DNA/RNA™ Water Kit and other commercial kits. Eluates were diluted ten-fold to determine inhibition levels. All samples analyzed using R3013 *Quick* SARS-CoV-2 Multiplex kit.



Sample Preparation Overview



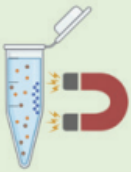
Liquid sample from collection site

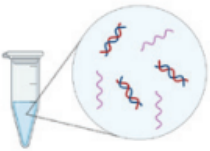
Sample Preparation (I)
Centrifuge then treat with
Wastewater Stabilization Buffer

Sample Lysis (II)
Homogenize using ZR
BashingBead™ Lysis Tubes.
(Recommended for complete microbial lysis)

Extraction (III)
Utilizing Zymo-Spin™ technology

Inhibitor Removal (IV)
Featuring MAGicBead™ technology





DNA & RNA is ready for any
downstream application

Protocol

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

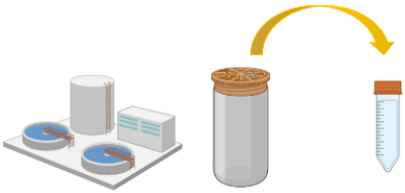
Buffer Preparation

- ✓ 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).

Liquid Samples (raw wastewater, sewage, natural[1] and finished[1] water)

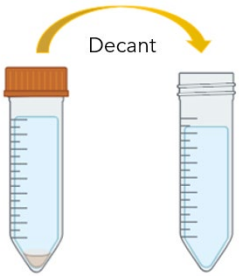


Liquid sample from collection site

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

Centrifuge to pellet debris.

2. Centrifuge at 4,000 *x g* for 2 minutes to pellet debris.[2]



Save the supernatant!

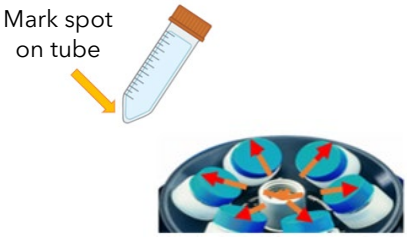
3. Carefully decant and transfer supernatant to new conical tube. **Save the supernatant!**

Note: Ensure supernatant is transferred to a conical tube compatible with floor model centrifuge.

[1] For low biomass liquid samples such as natural and finished water, proceed directly to Step 4 of (I) Sample Preparation.
[2] Centrifugation speed and time should be optimized based on sample turbidity.

≤50 mL liquid sample

4. Add 0.1 volume of **Wastewater Stabilization Buffer** to the liquid sample. Mix well by vortexing. Incubate at room temperature for 10 minutes. Centrifuge at 10,000 *x g* for 20 minutes.

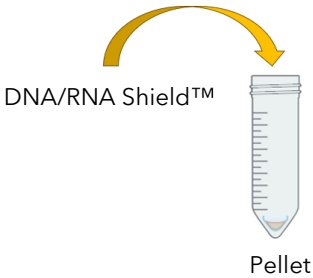


Place tube with mark facing outward on the rotor.

5. Mark a location near the bottom of the tube and orient outward on the rotor during centrifugation. This will aid in resuspension of pellets that may not be clearly visible.

6. 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.



7. Resuspend the pellet with 250 µl **DNA/RNA Shield™** (1X concentrate).[1] 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.*

[1] For viral enrichment, see Appendix.

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).
3. Proceed to Step 2 of **(II) Sample Lysis** on page 9.

Wastewater Solids and Sludge

1. Transfer up to 250 mg directly into **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)**.
2. Add 750 µl **DNA/RNA Shield**™ (1X concentrate).
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. Proceed with **(II) Sample Lysis** on page 9.

Ceres 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. 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.

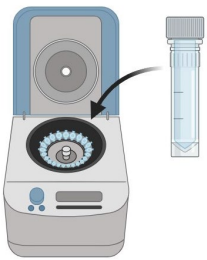
Transfer sample to ZR BashingBead™ Lysis Tube

1. Transfer up to 250 µl of the sample to **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)**. Add 750 µl **DNA/RNA Shield** (1X concentrate) to the tube and cap tightly.

Homogenize

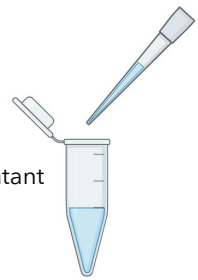
2. Secure prepared lysis tube in bead beater fitted with 2 ml tube holder assembly and process using optimized beat beating conditions (speed and time) for your device (see Appendix).

   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 2 volumes 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 by pipetting up and down.

5. Add 400 µl ethanol (95-100%) to this mixture from step 4 and mix well.

6. Transfer the mixture from step 5 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.

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

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

(IV) Inhibitor Removal

- ✓ Perform all steps at room temperature (15-30°C).
- ✓ For all buffer additions, mix well by pipetting up and down and/or by vortexing for 1-2 seconds, unless specified.
- ✓ This section of the protocol is compatible with automated platforms. See appendix.

1. Add 600 µl **Inhibitor Removal Binding Buffer** to the eluate (containing DNA and/or RNA) and mix well.

2. Add 30 µl **MAGicBead™ Mix** and mix well.

   Important: Before dispensing, completely resuspend **MAGicBead™ Mix** by vortexing and inverting vigorously until beads are uniformly distributed.

3. Incubate at room temperature with constant agitation[1] using a rotator (recommended ~30 rpm) for 5 minutes.

4. After removing the sample from the rotator, flick down or quick spin tube for 1-2 seconds to move residual buffer to the bottom of the tube. Carefully open the cap prior to applying it on magnetic stand.

5. Apply sample to magnetic stand until beads are fully pelleted. Carefully discard the supernatant, then remove the sample from magnetic stand.

6. Add 200 µl **Inhibitor Removal Wash Buffer** and mix well.

7. Apply sample to a magnetic stand until beads are fully pelleted. Carefully remove and discard the supernatant, then remove the sample from magnetic stand[2].

   Important: Ensure all residual wash buffer is removed, using a micropipette as needed.

8. Repeat Steps 6-7 for a total of 2 washes.

9. Add 50-100 µl **Inhibitor Removal Elution Buffer** and mix well by pipetting.

10. Incubate at room temperature for 5 minutes off the magnetic stand. Apply sample to magnetic stand until beads are fully pelleted.

11. Carefully transfer the eluate (containing DNA and/or RNA) to a nuclease-free tube.

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

[1] Beads settle quickly and should be well mixed with sample lysate just prior to incubation. Instead of a rotator, vortex or rollers can be used at moderate speed that will keep beads resuspended during incubation.
[2] Air-drying the beads prior to adding elution buffer is not necessary.

Appendices

Sample Collection

For water samples collected from autosampler, or water grab devices, **Wastewater Stabilization Buffer** is recommended for sample preservation. The addition of Wastewater Stabilization at the point of collection will best prevent further degradation of nucleic acids and reduce potential health risks.

1. For stabilization of DNA/RNA and pathogen inactivation, add 0.1 volume of **Wastewater Stabilization Buffer** to liquid samples at the time of collection and mix well. For high turbidity water samples, it is recommended to perform Steps 1-3 of **(I) Sample Preparation** prior to the addition of **Wastewater Stabilization Buffer.**

   Sample can be stored at room temperature for up to 1 week or frozen at ≤-70°C for long term storage.

2. When ready to process, aliquot 50 mL into a conical tube that is compatible with a floor model centrifuge.

3. Proceed with centrifugation in Step 4 of **(I) Sample Preparation** on page 7. No additional Wastewater Stabilization Buffer is required.

Viral Enrichment

✓ To enrich for viruses, a viral enrichment can be performed.

1. After completing Steps 1- 7 of **(I) Sample Preparation**, incubate the resuspended sample for 15 minutes at room temperature.

2. Centrifuge at 16,000 *x g* for 1 minute.

3. Transfer supernatant to new DNase/RNase-Free tube.

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

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] (reconstitute 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**.

Automation Scripts

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.

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 **RNA Wash Buffer** concentrate (R1003-3-24).

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

Product Description	Catalog No.	Amount
***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
DNase I Set	E1010 E1011 E1012	250 U 1500 U 5 x 1500 U
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000
ZR Lysis BashingBead™ Tubes (0.1 & 0.5 mm)	S6012-50	50

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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