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ZYMO RESEARCH

RNA Purification Made Simple™

Zymo Environ™ Water RNA Kit

Isolate inhibitor-free RNA from water

Highlights

- Enrich total RNA from large volumes (≤ 5 ml) and transform into small sample pellets for easy processing.
- Inactivates infectious agents and pathogens (viruses, bacteria, fungi, and parasites) for safe handling using DNA/RNA Shield™.
- Remove PCR inhibitors in one spin and elute concentrated RNA in ≥6 µl. Ready for any downstream application including RT-qPCR, RT-ddPCR, NGS.

Catalog Number:
R2042

Table of Contents

Product Contents

Zymo Environ™ Water RNA Kit	R2042 50 preps.	Storage Temp.
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	50	Room Temp.
DNA/RNA Shield™	50 ml	Room Temp.
Water Concentrating Buffer™[1]	2x 8 ml	Room Temp.
RNA Binding Buffer	50 ml	Room Temp.
RNA Prep Buffer	2x 25 ml	Room Temp.
RNA Wash Buffer[2]	24 ml	Room Temp.
DNase/RNase-Free Water	6 ml	Room Temp.
Prep Solution	30 ml	Room Temp.
Zymo-Spin™ IC Columns	50	Room Temp.
Zymo-Spin™ IIICG Columns	2x 50	Room Temp.
Zymo-Spin™ III-HRC Columns	50	Room Temp.
Collection Tubes	200	Room Temp.
Instruction Manual	1 pc	-

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:
1 Enough Water Concentrating Buffer™ is provided to process ~4 mL sample volume per prep.
2 Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.

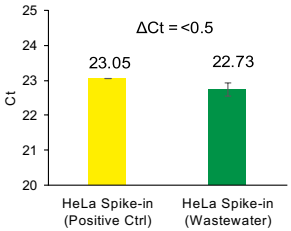
Specifications

- **Sample Sources –** Water, wastewater, sludge, sewage, biofilms, *etc.*
- **Sample Inactivation –** DNA/RNA Shield™ lyses cells, inactivates nucleases and infectious agents, and is ideal for safe sample storage and transport at ambient temperatures.
- **Sample Size –** Up to 5 ml of sample. The recommended processing volume is 4 ml or less.
- **Format –** Physical lysis using bead beating follow by spin-column purification.
- **RNA Purity –** High quality RNA ($A_{260}/A_{280} > 1.8$, $A_{260}/A_{230} > 1.8$) suitable for all downstream applications including RT-qPCR, RT-ddPCR, and NGS.
- **Yield –** Up to 10 µg RNA can eluted into ≥ 6 µl RNase-free water allowing for a highly concentrated sample.
- **RNA Storage –** RNA is eluted with 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, floor model centrifuge capable of spinning 50 ml conical tubes (optional), cell disrupter/pulverizer (optional).

Product Description

The **Zymo Environ™ Water RNA Kit** simplifies water surveillance by facilitating viral/microbial enrichment, inactivating pathogens, and improving limits of detection. The use of Water Concentrating Buffer (WCB) promotes the pelleting of viral capsids and protein-associated RNA, increasing SARS-CoV-2 RNA yields by more than 8-fold (Figure 1). DNA/RNA Shield™ allows for safe handling as it has been validated for inactivation of pathogens including SARS-CoV-2. Finally, the workflow yields highly concentrated, PCR inhibitor-free RNA (Figures 2 & 3) ready for downstream applications including RT-qPCR, RT-ddPCR, and NGS.

Figure 1. The Zymo Environ™ Water RNA Kit includes Water Concentrating Buffer™ (WCB). 5 ml of SARS-CoV-2 positive wastewater was processed with and without WCB (n=2). RT-qPCR showed the addition of WCB shifted average Ct values earlier by ~3 (an 8-fold increase in viral recovery).



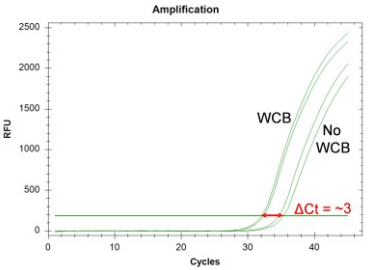
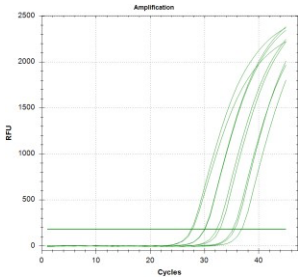


Figure 2. Extracted Wastewater RNA is free of PCR Inhibitors. 50K HeLa cells were spiked into DNA/RNA Shield™ (positive extraction control) and into wastewater RNA was extracted (n=2) and RT-qPCR (Quasar 670) analysis showed the ΔCt is <0.5, indicating the extraction system effectively removes inhibitors in wastewater. Samples were compared to a plate control containing the theoretical spike-in amount of RNA which amplified at 23.01 Ct.



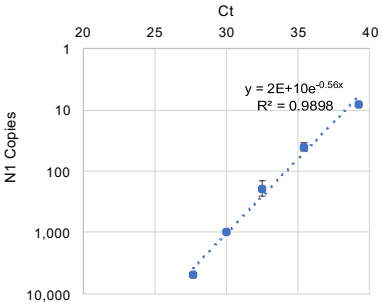


Figure 3. Improved Limit of Detection (LOD). SARS-CoV-2 sample was serially diluted from 5000 down to 8 copies and spiked into proxy wastewater and RNA was extracted (n=2). RT-qPCR targeting the N1 gene showed the dilution is linear (R^2 = 0.9898) and the workflow was able to achieve an LOD of 8-40 copies.

Protocol

The protocol consists of Buffer Preparation, Viral Enrichment, Sample Homogenization, and RNA Purification.

Buffer Preparation

✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate.

Viral Enrichment (Optional)[1]

1. Add 70 µl of **Water Concentrating Buffer**™ for every 1 mL of liquid sample. Mix well by vortexing. Let the sample sit for 10 minutes at room temperature.

2. Centrifuge at 3,000 *x g* for 15 minutes at room temperature.

3. Without disturbing the pellet (may not be visible), slowly decant or pipette out the supernatant leaving behind ~250 µl of pellet. Proceed to Sample Homogenization.

Sample Homogenization

1. Add 750 µl of **DNA/RNA Shield**™ to the above pellet or to 250 µl liquid sample to obtain a total of 1 ml of mixture. Mix well by vortexing.

2. Add the 1 ml mixture to a **ZR BashingBead**™ **Lysis Tube**.

3. Secure in a bead beater fitted with a 2 ml tube holder assembly and process[2].

4. Centrifuge the tube at 12,000 *x g* for 2 minutes to reduce foam.

5. Transfer 400 µl of the supernatant[3] into an RNase-free tube. Proceed to RNA Purification.

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1 Viral enrichment is designed for wastewater only (not sludge). Sample volumes as low as 250 µl may be used without using any Water Concentrating Buffer. This may result in less viral capsid captured but will speed up the protocol. Skip to Sample Homogenization.

2 Processing times may be as little as 1 minute when using high-speed (force) cell disruptors (*e.g.* FastPrep®-24, Bertin Precellys Evolution, or similar); vortex genie at low speed for 5 minutes. See manufacturer's literature for operation information.

3 Sample (*i.e.,* supernatant) and reagent volumes in this protocol can be adjusted proportionally if needed. For example, up to 700 µl may be used to increase the amount of viral capsid captured. However, this may result in longer processing times.

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RNA Purification

✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

1. Add 1 volume of **RNA Binding Buffer** to the supernatant. Mix well.

2. Transfer the mixture into a **Zymo-Spin™ IIICG Column**[1] in a **Collection Tube** and centrifuge. Save the flow-through!

   Add 1 volume of ethanol (95-100%) to the flow-through in the **Collection Tube** from Step 2 and mix well by pipetting up and down.

4. Transfer the mixture into a new **Zymo-Spin™ IIICG Column**[1] in a **Collection Tube** and centrifuge[2]. Discard the flow-through.

5. Add 400 µl of **RNA Prep Buffer** to the column and centrifuge. Then transfer the column into an RNase-free tube (not provided).

6. Add 100 µl of **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

7. Place a **Zymo-Spin™ III-HRC Filter** into a new Collection Tube and add 600 µl of **Prep Solution**. Centrifuge at 8,000 *x g* for 3 minutes and discard the flow-through.

8. Transfer the eluted RNA from step 6 into a prepared Zymo-Spin™ III-HRC Filter in an RNase-free tube and centrifuge at exactly 16,000 *x g* for 3 minutes.

9. Add 200 µl of **RNA Binding Buffer** to the filtrate and mix well by pipetting up and down.

10. Add 300 µl of ethanol (95-100%) and mix well by pipetting up and down.

11. Transfer the mixture into a **Zymo-Spin IC Column** in a **Collection Tube** and centrifuge. Discard the flow-through.

12. Add 400 µl of **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.

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1 To process samples > 800 µl, reload the column.
2 To process large volumes, a vacuum manifold may be used instead. To facilitate sample loading, 25 mL Reservoirs (C1039-25) may be purchased separately and used.

13. Add 700 µl of **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.

14. Add 400 µl of **RNA Wash Buffer** to the column and centrifuge for 2 minutes to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube.

15. Add 15 µl of **DNase/RNase-Free Water**[1] directly to the column matrix and centrifuge. The eluted RNA can be used immediately or stored at ≤ -70°C.

1 Alternatively, for highly concentrated RNA use ≥6 µl elution.

Ordering Information

Product Description	Catalog No.	Amount
Zymo Environ™ Water RNA Kit	R2042	50 preps.

Individual Kit Components	Catalog No.	Amount
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	S6012-50	50
DNA/RNA Shield™	R1100-50 R1100-250	50 ml 250 ml
Water Concentrating Buffer™	R2042-1-8 R2042-1-140	8 ml 140 ml
RNA Binding Buffer	R1013-2-25 R1013-2-50 R1013-2-100 R1013-2-1000	25 ml 50 ml 100 ml 1000 ml
RNA Prep Buffer	R1060-2-10 R1060-2-25	10 ml 25 ml
RNA Wash Buffer (concentrate)	R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48	6 ml 12 ml 24 ml 48 ml
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10	1 ml 4 ml 6 ml 10 ml
Zymo-Spin™ IC Columns	C1004-50 C1004-250	50 250
Zymo-Spin™ IIICG Columns	C1006-50-G C1006-250-G	50 250
OneStep™ PCR Inhibitor Removal Kit	D6030	50 preps.
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000

Notes

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