	Ethanol residue in the elute	Be sure to completely dry the column before elution
Little or no supernatant after initial centrifuge step	Insufficient centrifugal force	Check the centrifugal force and increase the centrifugal time if necessary
sample can not pass through the column	Clogging column	Check the centrifugal force and increase the time of centrifugation

If the above suggestions fail to resolve any problems you are having with the E.Z.N.A.® Water DNA Kit, please feel free to fax our technical specialists at:

US customers: 800-832-8896 or 770-931-8400
All other customers: (770) 931-0230
Or direct your questions via E-mail to info@omegabiotek.com.

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18. **Place the column in to a new 2 ml collection tube (supplied). Add 750µl DNA Wash Buffer (diluted with ethanol) and centrifuge at 10,000 x g for 30 seconds. Discard the flow-through liquid and re-use the collection tube.**

    Note: DNA Wash Buffer has to be diluted with ethanol before use, see dilution instruction on page 3.

19. **Place the empty column back into the collection tube and spin at maximum speed (≥14,000 x g) for 2 minutes to dry the column.**

    Note: This step is critical to remove any trace of ethanol residue from DNA wash Buffer that might interfere downstream enzymatic applications.

20. **Place the HiBind® DNA column into a new 1.5 ml centrifuge tube.** Apply 50-100µl of DNA Elution Buffer to the center of the membrane in the column. Incubate at 65°C for 3 minutes.

21. **Centrifuge at maximum speed for 1 minute to elute DNA.**

22. **Store the elute DNA sample at -20°C.**

Kit Contents

Product Number	D5525-00	D5525-01	D5525-02
Purification times	5 Preps	50 Preps	200 Preps
HiBind® DNA Columns	5	50	200
2 mL Collection Tubes	10	100	400
Glass Beads	2.7 g	27 g	120 g
HTR Reagent	1 mL	10 mL	40 mL
Buffer SLX	18 mL	180 mL	3 x 220 mL
SP2 Buffer	6 mL	60 mL	220 mL
XP1 Buffer	5 mL	40 mL	180 mL
Elution Buffer	5 mL	30 mL	100 mL
DNA Wash Buffer	2 mL	20 mL	2 × 50 mL
Instruction Booklet	1	1	1

Materials to be provided by user

- Microcentrifuge capable of at least 14,000 x g
- table top centrifuge capable of 4000 x g.
- adapter for 50 ml centrifuge tube.
- Nuclease-free 1.5 mL or 2 mL microfuge tubes
- Nuclease-free 50 ml centrifuge tube capable of 4000 x g.
- Water bath equilibrated to 65ºC
- Absolute (96%-100%) ethanol
- RNase A stock solution at 25 mg/mL

Introduction

The E.Z.N.A.® Water DNA Kit allows rapid and reliable isolation of high- quality DNA from various micro organisms in water samples. The system combines the reversible nucleic acid-binding properties of HiBind® matrix with the speed and versatility of spin column technology to eliminate PCR inhibiting compounds from water sample. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

Overview

If using the E.Z.N.A.® Water DNA Kit for the first time, please read this booklet to become familiar with the procedure. Water sample is first filtered using microporous filter. The filter is then put into a tube contains beads and buffer to homogenize and lysis the sample. Humic acid, proteins, polysaccharides, and other contaminants are subsequently precipitated after a heat-frozen step. Contaminants are further removed by a special HTR reagent treatment. Binding conditions are then adjusted and the sample is applied to an HiBind® DNA spin-column. Two rapid wash steps remove trace contaminants and pure DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

All components of the E.Z.N.A.® Water DNA Kit should be stored at 22°C-25°C. Under this storage conditions, DNA has successfully been purified and used for PCR after 24 months of storage. During shipment, or storage in cool ambient conditions, precipitates may form in some buffers. It is possible to dissolve such deposits by incubation the solution at 65°C.

Troubleshooting Guide

Problem	Cause	Suggestions
A260/230 ratio is low	inefficient elimination of inhibitory compounds	Repeat the DNA isolation with a new sample, be sure to mix the sample with HTR Buffer throughly.
	No ethanol added to the lysate before loading to the column	Repeat the DNA isolation with a new sample.
	DNA wash Buffer prepared with lower percentage ethanol	prepare DNA Wash Buffer with 96-100% ethanol
A260/280 ratio is high	RNA contamination	Be sure to treat the sample with RNase A in step 9.
Low DNA yield or no DNA eluted	Sample stored incorrectly	Sample should be store at 4°C or -20°C
	Poor homogenization of sample.	Repeat the DNA isolation with a new sample, be sure to mix the sample throughly.
	Incorrect XP1 Buffer was added before loading to the column	Repeat the DNA isolation with a new sample
	DNA washed off.	Dilute Wash Buffer Concentrate by adding appropriate volume of absolute ethanol prior to use (page 3).
Problems in downstream applications	Too much DNA inhibit PCR reaction	Diluted the DNA elute used in the downstream application if possible.
	No-specific bands in downstream PCR	Use hot-start Taq polymerase mixture
	inhibitory substance in the eluted DNA.	Check the A260/230 ration Diluted the elute to 1:50 if necessary

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.® Water DNA Kit protocol.
- Dilute DNA Wash Buffer Concentrate with absolute ethanol as follows and store at room temperature.

D5525-00	Add 8 mL (96%-100%) ethanol.
D5525-01	Add 80 mL (96%-100%) ethanol to each bottle.
D5525-02	Add 200 mL (96%-100%) ethanol to each bottle.

- Preheat Elution Buffer at 65ºC

Water DNA Isolation Protocol

1. **Filter the water samples using a microporous filter paper (0.22 μm or 0.45 μm).** The volume of water can be used depend on the microbial load and turbidity of the water sample. For turbid water sample, it is highly recommended to use a prefilter paper to prevent clogging of the microporous filter.

2. **Take the filter membrane from the filter adapter and scissor the membrane for quartering.** Insert the filter membrane to an ascetical 50 ml centrifuge tube.

3. **Add 3 ml SLX Buffer and 500 mg glass beads (suppplied) to the tube.**

4. **Vortex at maximum speed for 3 minutes or until the sample is throughly homogenized.**

5. **Incubate at 70ºC or 90ºC (difficult lysed bacteria) for 10 min. Mix sample 2-3 times during incubation by vortexing the tube**.

6. **Add 1 ml of SP2 Buffer the tube and mix throughly by vortexing the tube for 30 seconds**. Incubate on ice for 5 minutes.

7. **Centrifuge at 4,000 x g for 10 minutes at 4ºC.**

8. **Transfer the cleared supernatant to a new 15 or 50 ml tube and add 0.7 volume of isopropanol.** Mix throughly by inverting the tube 30-50 times. Incubate at -20ºC for >30 minutes.

9. **Centrifuge at 4,000 x g for 20 minutes at 4ºC to pellet the DNA.**

10. **Carefully remove and discard the supernatant, make sure not disturb the DNA pellet.**

11. **Add 400 μl Elution Buffer to the tube and mix throughly by vortexing for 20 seconds.** Incubate at 65○C for 10-30 minutes to dissolve the DNA.

    Note: If RNA-free DNA is desired, add 10μl of RNase A (25mg/ml) in to the sample at this point.

12. **Transfer the sample to a new 1.5 ml micrtube and add 100μl HTR Reagent.** Vortex the tube for 10 seconds to mix . Incubate 2 minutes at room temperature.
    Note: Vortex the bottle contains HTR Reagent to completely resuspend the HTR Reagent before use.

13. **Centrifuge at 14,000 x g for 3 minutes to pellet the HTR Reagent.**

    Note: If the sample still show brow or dark color after the HTR extraction. Perform HTR extraction by repeat step 12-13.

14. **Transfer the cleared supernatant (normally 400 μl) to a new 1.5 ml microtube. Add Equal volume of Buffer XP1. Mix throughly by voretxing.**

15. **Place a HiBind® DNA column into a 2 ml collection tube (supplied).**

16. **Apply entire sample to a HiBind® DNA column inserted in a 2 ml collection tube. Centrifuge at 10,000 x for 1 minute at room temperature.** Discard the flow-through and re-use the collection tube in next step.

17. **Add 300μl of Buffer XP1 to the column. Centrifuge at 10,000 x g for 1 minute at room temperature.** Discard the flow-through liquid and the collection tube.