E.Z.N.A.® Water DNA Kit

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Manual Revision: April 2017

OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction and Overview

The E.Z.N.A.® Water DNA Kit allows rapid and reliable isolation of high-quality DNA from various microorganisms 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 applications. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

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 containing the beads and buffer to homogenize and lysis the sample. Humic acid, proteins, polysaccharides, and other contaminants are subsequently precipitated after a heat-freeze step. Contaminants are further removed by a special cHTR Reagent treatment. Binding conditions are then adjusted and the sample is transferred to a HiBind® DNA Mini 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.

New in this Edition:

April 2017:

- This manual has been edited for content. Instructions for addition of 2-mercaptoethanol has been clarified.

December 2016:

- cHTR Reagent has replaced HTR Reagent.

May 2014:

- Glass Beads are now called Glass Beads X. This is a name change only. The glass beads have not changed.

July 2013:

- This manual has been edited for content and redesigned to enhance user readability.
- Equilibration Buffer is no longer included with this kit. An optional Column Equilibration Protocol has been added to the protocol for your convenience.
- Equilibration Buffer is replaced with 3M NaOH provided by the user.

Kit Contents

Product	D5525-00	D5525-01	D5525-02
Purifications	5	50	200
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
Glass Beads X	2.7 g	27 g	120 g
cHTR Reagent	1 mL	10 mL	40 mL
SLX-Mlus Buffer	18 mL	180 mL	3 x 220 mL
P2 Buffer	6 mL	60 mL	220 mL
DS 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	1.5 mL	15 mL	3 x 25 mL
User Manual	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® Water DNA Kit components are guaranteed for at least 24 months from the date of purchase when stored as follows. cHTR Reagent should be stored at 2-8˚C. All other materials should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits(except cHTR Reagent) by warming the solution at 37°C and gently shaking.

Preparing Reagents

1. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D5525-00	6 mL
D5525-01	60 mL
D5525-02	100 mL

2. Add 10 µL 2-mercaptoethanol per 1 mL SLX-Mlus before use.

E.Z.N.A.® Water DNA Mini Kit Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 14,000 x *g*
- Centrifuge capable of 4,000 x *g* with adaptor for 50 mL centrifuge tubes
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Nuclease-free 50 mL centrifuge tubes capable of 4,000 x *g*
- Water baths capable of 70°C
- Ice bucket
- 0.22 µm or 0.45 µm microporous filter paper
- 2-mercaptoethanol
- 100% ethanol
- 100% isopropanol
- Optional: RNase A (25 mg/mL)
- Optional: 3M NaOH

Before Starting:

- Heat water bath to 70°C
- Heat Elution Buffer to 65°C
- Prepare DNA Wash Buffer and SLX-Mlus Buffer according to the instructions in the Preparing Reagents section on Page 4

1. Filter the water samples using microporous filter paper (0.22 µm or 0.45 µm).

   Note: The volume of water used depends on the microbial load and turbidity of the water sample. For turbid water samples, it is highly recommended to use a prefilter paper to prevent clogging of the microporous filter.

2. Remove the filter from the adapter, cut the membrane into four pieces, and place the filter in a clean 50 mL centrifuge tube.

3. Add 3 mL SLX-Mlus Buffer and 500 mg Glass Beads X.

   Note: SLX-Mlus Buffer must be mixed with 2-mercaptoethanol prior to use. Please see Page 4 for instructions.

4. Vortex at maximum speed for 5-10 minutes or until the sample is thoroughly homogenized.

5. Add 1 mL DS Buffer.

6. Incubate at 70°C for 10 minutes. Vortex the sample 2-3 times during incubation.

7. Add 1 mL P2 Buffer. Vortex for 30 seconds to mix thoroughly.

8. Let sit on ice for 5 minutes.

9. Centrifuge at 4,000 x *g* for 10 minutes at room temperature.

10. Transfer the cleared supernatant to a new 50 mL centrifuge tube.

11. Add 0.7 volumes isopropanol. Invert the tube 20 times to mix thoroughly.

12. Centrifuge at 4,000 x *g* for 10 minutes at room temperature.

13. Carefully aspirate and discard the supernatant. Do not disturb the DNA pellet.

14. Add 400 µL Elution Buffer. Vortex for 20 seconds to mix thoroughly.

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

15. Incubate at 65°C for 10 minutes to dissolve the DNA.

16. Transfer the sample to a new 1.5 mL microcentrifuge tube.

17. Add 100 µL cHTR Reagent. Vortex for 10 seconds to mix.

    Note: Vortex the cHTR Reagent bottle before use to completely resuspend the cHTR Reagent.

18. Let sit for 2 minutes at room temperature.

19. Centrifuge at 14,000 x *g* for 3 minutes.

    Note: Repeat steps 17-19 if the sample still appears brown or dark in color.

20. Transfer the cleared supernatant (normally 400 µL) to a new 1.5 mL microcentrifuge tube.

21. Add equal volume XP1 Buffer. Vortex to mix thoroughly.

22. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube (supplied).

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Centrifuge at maximum speed for 60 seconds.
3. Discard the filtrate and reuse the collection tube.

23. Transfer the entire sample from Step 21 to the HiBind® DNA Mini Column.

24. Centrifuge at 10,000 x *g* for 1 minute at room temperature.

25. Discard the filtrate and reuse the collection tube.

26. Add 300 µL XP1 Buffer.

27. Centrifuge at 10,000 x *g* for 1 minute at room temperature.

28. Discard the filtrate and reuse the collection tube.

29. Transfer the HiBind® DNA Mini Column to a new 2 mL Collection Tube.

30. Add 750 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

31. Centrifuge at maximum speed for 1 minute.

32. Discard the filtrate and reuse the collection tube.

Optional: Repeat Steps 30-32 for a second DNA Wash Buffer wash step.

33. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

    Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

34. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

35. Add 50-100 µL Elution Buffer or sterile deionized water directly to the center of the column membrane.

    Note: The efficiency of eluting DNA from the HiBind® DNA Mini Column is dependent on pH. If using sterile deionized water, make sure that the pH is around 8.5.

36. Incubate at 65°C for 5 minutes.

37. Centrifuge at maximum speed for 1 minute.

    Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

38. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Absorbance of purified DNA does not accurately reflect quantity of the plasmid (A_{260}/A_{280} ratio is high or low)	
Inefficient elimination of inhibitory compounds	Repeat DNA isolation with a new sample, making sure to mix the sample with cHTR Reagent thoroughly.
DNA Wash Buffer is diluted with ethanol containing impurities	Check the absorbance of the ethanol between 250 nm and 300 nm. Do not use ethanol with high absorbance. Traces of impurities may remain on the binding column after washing and contribute to the absorbance in the final product.
RNA contamination	Add RNase A (25 mg/mL) at Step 14.
Low DNA yield or no DNA eluted	
Sample stored incorrectly	Sample should be stored at 4°C or -20°C.
Poor sample homogenization	Repeat the DNA isolation with a new sample, be sure to mix the sample thoroughly.
Incorrect XP1 Buffer was added before loading to the column	Repeat the DNA isolation with a new sample.
DNA washed off	Prepare DNA Wash Buffer according to instructions on Page 4.
Problems in downstream applications	
Too much DNA inhibit PCR reaction	Dilute the DNA elute used in the downstream application if possible.
Non-specific bands in downstream PCR	Use hot-start Taq polymerase mixture.
Inhibitory substance in the eluted DNA	Check the A_{260}/A_{230} ratio.
Ethanol residue in the elute	Dilute the elute to 1:50 if necessary.
Sample can not pass through the column	
Insufficient centrifugal force	Check the centrifugal force and increase the centrifugal time if necessary.
Clogged column	Check the centrifugal force and increase the time of centrifugation.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Elution Buffer, 100 mL	PDR048
DNA Wash Buffer, 100 mL	PDR044
RNase A, 400 µL	AC117
cHTR Reagent, 50 mL	PD089

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