omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Universal Pathogen Kit

D4035-00	5 preps
D4035-01	50 preps

Manual Date: April 2024
Revision Number: v2.4

For Research Use Only

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Introduction

The E.Z.N.A.® Universal Pathogen Kit allows rapid and reliable isolation of high-quality host genomic DNA, gram positive and negative bacterial DNA, fungal spore DNA, and viral DNA and RNA from tissue, urine, serum, and fecal samples. Elution volumes as low as 15 µL can be used to maintain higher nucleic acid concentrations.

The system combines the Omega Bio-tek's MicroElute® LE DNA Columns with RBB Buffer to eliminate PCR inhibiting compounds within the samples and elute highly concentrated DNA. Purified DNA is suitable for PCR, restriction digestion, and hybridization applications. There are no organic extractions thus reducing plastic waste and hands-on time and multiple samples can be processed in parallel.

New in this Edition:

April 2024
- Addition of Warnings and Safety Information.

October 2022
- The column equilibration step was moved within the Tissue Protocol

September 2022
- Added column equilibration protocol for more consistent results.

Kit Contents

Product Number	D4035-00	D4035-01
Purifications	5 preps	50 preps
Disruptor Tubes	5	50
MicroElute® LE DNA Columns	5	50
2 mL Collection Tubes	10	100
SLX-Mlus Buffer	5 mL	50 mL
DS Buffer	500 µL	4 mL
PCP Buffer	1.2 mL	12 mL
RBB Buffer	4 mL	40 mL
HBC Buffer	3 mL	21 mL
DNA Wash Buffer	1.5 mL	15 mL
Elution Buffer	2 mL	15 mL
Proteinase K Solution	150 µL	1.5 mL
User Manual	✓	✓

Storage and Stability

All of the E.Z.N.A.® Universal Pathogen Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Proteinase K Solution can be stored at room temperature for up to 6 months. For long-term storage, store Proteinase K Solution at 2-8°C. Store all other components at room temperature and away from bright light. During shipment or storage in cool ambient conditions, precipitates may form in some of the buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Warnings

This kit is for research use only.

Please read all instructions carefully before using the kit.

Decontaminate and dispose of all potentially infectious materials in accordance with applicable local, state, and national regulations. Please refer to safety data sheets (SDSs) for information on disposal of different components included in this kit.

Safety Information

All chemicals and biological materials are potentially hazardous. Biological samples such as plasma, serum, tissues, body fluids, blood etc. are potentially infectious and must be treated as biohazardous materials. Conduct all work in properly equipped facilities following universal precautions and using appropriate personal safety equipment such as disposable gloves, lab coats, safety glasses etc. as required by policies and procedures outlined by your facility. Please refer to safety data sheets (SDSs) for information on safe handling, transport and disposal of different components included in this kit. SDSs are made available in PDF format on the product page at www.omegabiotek.com. Discard all waste in accordance with the local safety regulations.

Some of the buffers included in the product contain guanidine-based chaotropic agents, which can form highly reactive compounds when combined with bleach. DO NOT add bleach or acidic solutions to guanidine-containing waste. Please access the SDSs online for detailed information on the reagents.

Preparing Reagents

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

Kit	100% Ethanol to be Added
D4035-00	6 mL
D4035-01	60 mL

2. Dilute HBC Buffer with 100% isopropanol follows and store at room temperature.

Kit	100% Isopropanol to be Added
D4035-00	1.2 mL
D4035-01	8.4 mL

Tissue Protocol

Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 12,000*g*
- Incubator capable of 70°C
- Centrifuge tubes with a capacity of at least 1.7 mL
- Nuclease-free 1.5 mL centrifuge tubes for DNA storage
- Vortexer
- 100% ethanol
- 100% isopropanol
- 3M NaOH
- Sterile deionized water
- Optional: Mixer mill such as a SPEX CertiPrep Geno/Grinder® 2010 or Qiagen TissueLyser

Before Starting:

- Prepare HBC Buffer and DNA Wash Buffer according to the "Preparing Reagents" section on Page 5.
- Set an incubator to 70°C.
- Heat Elution Buffer to 70°C.

1. Briefly spin the Disruptor Tube to remove any glass beads from the wall of the tube. Uncap the Disruptor Tube and save the cap for use in Step 3.

2. Add 25-30 mg tissue.

3. Add 725 µL SLX-Mlus Buffer. Seal the Disruptor Tube with the cap removed in Step 1.

4. Vortex at maximum speed for 3-5 minutes to lyse and homogenize the samples. For best results, a Mixer Mill, such as Spex CertiPrep Geno/Grinder® 2010 or Qiagen Tissuelyser, should be used.

   Note: Depending on the sample type and amount, the volume of SLX-Mlus Buffer may need to be adjusted so that 300 µL can be recovered during Step 12.

5. Centrifuge at 1,000-2,000*g* for 15 seconds at room temperature.

6. Uncap the Disruptor Tube and save the cap for use in Step 8.

7. Add 72 µL DS Buffer and 20 µL Proteinase K Solution.

8. Seal the Disruptor Tube with the cap removed in Step 6.

9. Vortex for 60 seconds to mix thoroughly.

10. Incubate at 70°C for 15 minutes. Mix once during incubation.

11. Centrifuge at 12,000*g* for 5 minutes.

12. Transfer 300 µL cleared supernatant to a 1.5 mL centrifuge tube (not provided).

    Note: Do not transfer any debris as it can reduce yield and purity.

13. Add 600 µL RBB Buffer. Vortex to mix thoroughly.

14. Let sit at room temperature for 5 minutes.

15. Insert a MicroElute® LE DNA Column into a 2 mL Collection Tube (provided) and follow the column equilibration steps listed below:

Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the MicroElute® LE DNA Column.
2. Centrifuge at 10,000*g* for 30 seconds.
3. Add 500 µL sterile deionized water to the MicroElute® LE DNA Column.
4. Centrifuge at 10,000*g* for 30 seconds.
5. Discard the filtrate and reuse the collection tube.

16. Transfer 700 µL sample from Step 14 to the MicroElute® LE DNA Column.

17. Centrifuge at maximum speed for 1 minute.

18. Discard the filtrate and reuse the collection tube.

19. Transfer the remaining lysate from Step 14 to the MicroElute® LE DNA Column.

20. Centrifuge at maximum speed for 1 minute.

21. Discard the filtrate and reuse the collection tube.

22. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 5 for instructions.

23. Centrifuge at maximum speed for 30 seconds.

24. Discard the filtrate and collection tube.

25. Insert the MicroElute® LE DNA Column into a new 2 mL Collection Tube.

26. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 5 for instructions.

27. Centrifuge at maximum speed for 30 seconds.

28. Discard the filtrate and reuse the collection tube.

29. Repeat Steps 26-28 for a second DNA Wash Buffer wash step.

30. Centrifuge the empty MicroElute® LE DNA Column at maximum speed for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications.

31. Transfer the MicroElute® LE DNA Column into a nuclease-free 1.5 mL microcentrifuge tube (not provided).

32. Add 15-100 µL Elution Buffer heated to 70°C.

33. Let sit at room temperature for 2 minutes.

34. Centrifuge at maximum speed for 1 minute.

35. Repeat Steps 32-34 for a second elution step.

    Note: Each 200 µL elution will typically yield of 60-70% of the DNA bound to the column. Two elutions will generally yield ~90%. However, increasing the elution volume will reduce the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using 50-100 µL Elution Buffer which slightly reduces overall DNA yield. Volumes lower than 50 µL greatly reduce yields. In some instances yields may be increased by incubating the column at 70°C (rather than room temperature) upon the addition of Elution Buffer.

36. Store eluted DNA at -20°C.

Serum & Stool Protocol

The following will isolate DNA and RNA from gram-positive & negative bacteria, fungal spores, and viruses. ***If only gram-negative bacteria or viral RNA & DNA is required, Steps 4-6 can be skipped.***

Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 12,000*g*
- Incubator capable of 70°C
- Centrifuge Tubes with a capacity of at least 1.7 mL
- 1.5 mL centrifuge tubes for DNA storage
- Vortexer
- 100% ethanol
- 100% isopropanol
- 3M NaOH
- Sterile deionized water
- Optional: Mixer mill such as a SPEX CertiPrep Geno/Grinder® 2010 or Qiagen TissueLyser

Before Starting:

- Prepare HBC Buffer and DNA Wash Buffer according to the "Preparing Reagents" section on Page 5.
- Set an incubator to 70°C.
- Heat Elution Buffer to 70°C.

1. Briefly spin the Disruptor Tube to remove any glass beads from the wall of the tube. Uncap the Disruptor Tube and save the cap for use in Step 3.

2. Add 250 µL stool sample or serum.

3. Add 475 µL SLX-Mlus Buffer. Seal the Disruptor Tube with the cap removed in Step 1.

   Note: If only gram-negative bacteria or viral RNA & DNA is required, Steps 4-6 can be skipped. Continue to Step 7 below.

4. Vortex at maximum speed for 3-5 minutes to lyse and homogenize samples. For best results, a Mixer Mill, such as Spex CertiPrep Geno/Grinder® 2010 or Qiagen Tissuelyser, should be used.

   Note: Depending on the sample type and amount, the volume of SLX-Mlus Buffer may need to be adjusted so that 300 µL can be recovered during Step 12.

5. Centrifuge at 1,000-2,000*g* for 15 seconds at room temperature.

6. Uncap the Disruptor Tube and save the cap for use in Step 8.

7. Add 72µL DS Buffer and 20 µL Proteinase K Solution.

8. Seal the Disruptor Tube with the cap removed in Step 6.

9. Vortex for 60 seconds to mix thoroughly.

10. Incubate at 70°C for 15 minutes. Mix once during incubation.

11. Centrifuge at 12,000*g* for 5 minutes.

12. Transfer 300 µL cleared supernatant to a 1.5 mL centrifuge tube (not provided)

    Note: Do not transfer any debris as it can reduce yield and purity.

13. Add 600 µL RBB Buffer. Vortex to mix thoroughly.

14. Let sit at room temperature for 5 minutes.

15. Insert a MicroElute® LE DNA Column into a 2 mL Collection Tube (provided) and follow the column equilibration steps listed below:

Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the MicroElute® LE DNA Column.
2. Centrifuge at 10,000 x *g* for 30 seconds.
3. Add 500 µL sterile deionized water to the MicroElute® LE DNA Column.
4. Centrifuge at 10,000 x *g* for 30 seconds.
5. Discard the filtrate and reuse the collection tube.

16. Transfer 700 µL sample from Step 14 to the MicroElute® LE DNA Column.

17. Centrifuge at maximum speed for 1 minute.

18. Discard the filtrate and reuse the collection tube.

19. Transfer the remaining lysate from Step 14 to the MicroElute® LE DNA Column.

20. Centrifuge at maximum speed for 1 minute.

21. Discard the filtrate and reuse the collection tube.

22. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 5 for instructions.

23. Centrifuge at maximum speed for 30 seconds.

24. Discard the filtrate and collection tube.

25. Insert the MicroElute® LE DNA Column into a new 2 mL Collection Tube.

26. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 5 for instructions.

27. Centrifuge at maximum speed for 30 seconds.

28. Discard the filtrate and reuse the collection tube.

29. Repeat Steps 26-28 for a second DNA Wash Buffer wash step.

30. Centrifuge the empty MicroElute® LE DNA Column at maximum speed for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications**.**

31. Transfer the MicroElute® LE DNA Column into a nuclease-free 1.5 mL microcentrifuge tube (not provided).

32. Add 15-100 µL Elution Buffer heated to 70°C.

33. Let sit at room temperature for 2 minutes.

34. Centrifuge at maximum speed for 1 minute.

35. Repeat Steps 32-34 for a second elution step.

    Note: Each 200 µL elution will typically yield of 60-70% of the DNA bound to the column. Two elutions will generally yield ~90%. However, increasing the elution volume will reduce the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using 50-100 µL Elution Buffer which slightly reduces overall DNA yield. Volumes lower than 50 µL greatly reduce yields. In some instances yields may be increased by incubating the column at 70°C (rather than room temperature) upon the addition of Elution Buffer.

36. Store eluted DNA at -20°C.

Urine & Blood Protocol

The following will isolate DNA and RNA from gram-positive & negative bacteria, fungal spores, and viruses. ***If only gram-negative bacteria and viral RNA & DNA is required Steps 4-6 can be skipped.***

Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 12,000*g*
- Incubator capable of 70°C
- Centrifuge Tubes with a capacity of at least 1.7 mL
- 1.5 mL centrifuge tubes for DNA storage
- Vortexer
- Ice bucket
- 100% ethanol
- 100% isopropanol
- 3M NaOH
- Sterile deionized water
- Optional: Mixer mill such as a SPEX CertiPrep Geno/Grinder® 2010 or Qiagen TissueLyser

Before Starting:

- Prepare HBC Buffer and DNA Wash Buffer according to the "Preparing Reagents" section on Page 5
- Set an incubator to 70°C
- Heat Elution Buffer to 70°C
- Prepare an ice bucket

1. Briefly spin the Disruptor Tube to remove any glass beads from the wall of the tube. Uncap the Disruptor Tube and save the cap for use in Step 3.

2. Add 250 µL urine or whole blood sample to the Disruptor Tube.

3. Add 275 µL SLX-Mlus Buffer. Seal the Disruptor Tube with the cap removed in Step 1.

   Note: If only gram-negative bacteria or viral RNA & DNA is required, Steps 4-6 can be skipped. Continue to Step 7 below.

4. Vortex at maximum speed for 3-5 minutes to lyse and homogenize samples. For best results, a Mixer Mill, such as Spex CertiPrep Geno/Grinder® 2010 or Qiagen Tissuelyser, should be used.

   Note: Depending on the sample type and amount, the volume of SLX-Mlus Buffer may need to be adjusted so that 300 µL can be recovered during Step 13.

5. Centrifuge at 1,000-2,000*g* for 15 seconds at room temperature.

6. Uncap the Disruptor Tube and save the cap for use in Step 8.

7. Add 50 µL DS Buffer and 20 µL Proteinase K Solution.

8. Seal the Disruptor Tube with the cap removed in Step 6.

9. Vortex for 60 seconds to mix thoroughly.

10. Incubate at 70°C for 15 minutes. Mix once during incubation.

11. Add 200 µL PCP Buffer. Let sit on ice for 5 minutes.

12. Centrifuge at 12,000*g* for 10 minutes.

13. Transfer 300 µL cleared supernatant to a 1.5 mL centrifuge tube (not provided).

    Note: Do not transfer any debris as it can reduce yield and purity.

14. Add 600 µL RBB Buffer. Vortex to mix thoroughly.

15. Let sit at room temperature for 5 minutes.

16. Insert a MicroElute® LE DNA Column into a 2 mL Collection Tube (provided) and follow the column equilibration steps listed below:

Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the MicroElute® LE DNA Column.
2. Centrifuge at 10,000*g* for 30 seconds.
3. Add 500 µL sterile deionized water to the MicroElute® LE DNA Column.
4. Centrifuge at 10,000*g* for 30 seconds.
5. Discard the filtrate and reuse the collection tube.

17. Transfer the 700 µL sample from Step 15 to the MicroElute® LE DNA Column.

18. Centrifuge at maximum speed for 1 minute.

19. Discard the filtrate and reuse the collection tube.

20. Transfer the remaining lysate from Step 15 to the MicroElute® LE DNA Column.

21. Centrifuge at maximum speed for 1 minute.

22. Discard the filtrate and reuse the collection tube.

23. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 5 for instructions.

24. Centrifuge at maximum speed for 30 seconds.

25. Discard the filtrate and collection tube.

26. Insert the MicroElute® LE DNA Column into a new 2 mL Collection Tube.

27. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 5 for instructions.

28. Centrifuge at maximum speed for 30 seconds.

29. Discard the filtrate and reuse the collection tube.

30. Repeat Steps 27-29 for a second DNA Wash Buffer wash step.

31. Centrifuge the empty MicroElute® LE DNA Column at maximum speed for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications**.**

32. Transfer the MicroElute® LE DNA Column into a nuclease-free 1.5 mL microcentrifuge tube (not provided).

33. Add 15-100 µL Elution Buffer heated to 70°C.

34. Let sit at room temperature for 2 minutes.

35. Centrifuge at maximum speed for 1 minute.

36. Repeat Steps 33-35 for a second elution step.

    Note: Each 200 µL elution will typically yield of 60-70% of the DNA bound to the column. Two elutions will generally yield ~90%. However, increasing the elution volume will reduce the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using 50-100 µL Elution Buffer which slightly reduces overall DNA yield. Volumes lower than 50 µL greatly reduce yields. In some instances yields may be increased by incubating the column at 70°C (rather than room temperature) upon the addition of Elution Buffer.

37. Store eluted 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**.

Problem	Cause	Solution
A_{260}/A_{230} ratio is low	Salt contamination	• Repeat the DNA isolation with a new sample. • Perform a second wash with HBC Buffer.
A_{260}/A_{280} ratio is high	RNA contamination	The protocol does not remove RNA. If desired, add 5 µL RNase A (25 mg/mL) after lysate is cleared and before binding buffers are added. Let sit at room temperature for 5 minutes.
Low DNA Yield or no DNA Yield	Poor homogenization of sample	Repeat the DNA isolation with a new sample, be sure to mix the sample with SLX-Mlus Buffer thoroughly. Use a commercial homogenizer if possible.
	DNA washed off	Make sure HBC Buffer is mixed with isopropanol and DNA Wash Buffer is mixed with ethanol.
	Column Equilibration not performed	Perform the column equilibration protocol as instructed in the manual for consistent results.
Problems in downstream applications	BSA not added to PCR mixture	Add BSA to a final concentration of 0.1 µg/mL to the PCR mixture.
	Too much DNA inhibits PCR reactions	Dilute the DNA elute used in the downstream application if possible.
	Non-specific bands in downstream PCR	Use hot-start Taq polymerase mixture.
Problems in downstream applications	Inhibitory substance in the eluted DNA	Check the A_{260}/A_{230} ratio. Dilute the elute to 1:50 if necessary.

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SPEX CertiPrep Geno/Grinder® 2010 or Qiagen TissueLyser are all trademarks of their respective companies.
PCR is a patented process of Hoffman-La Roche. Use of the PCR process requires a license.

Notices & Disclaimers

For European Union Use.

RBB Buffer contains Triton X-100, 2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol (CAS 9002-93-1), a substance included in the European Authorisation list (Annex XIV) of REACH Regulation (EC) No 1907/2006. Substances and mixtures used for the purpose of Scientific Research and Development (SR&D) are exempt from authorization requirements if used below 1 tonne per year in volume.

Scientific Research and Development includes experimental research or analytical activities at a laboratory scale such as synthesis and testing of applications of chemicals, release tests, etc. as well as the use of the substance in monitoring and routine quality control or in vitro diagnostics.

Notes:

For more purification solutions, visit **www.omegabiotek.com**

AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

SAMPLE TYPES


Blood / Plasma

Plasmid


Cultured Cells


Plant & Soil


NGS Clean Up


Tissue


FFPE


Fecal Matter

omega BIO-TEK

innovations in nucleic acid isolation

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