omega BIO-TEK

innovations in nucleic acid isolation

Product Manual

MicroElute® Cycle-Pure Kit

D6293-00	5 preps
D6293-01	50 preps
D6293-02	200 preps

MicroElute® Gel Extraction Kit

D6294-00	5 preps
D6294-01	50 preps
D6294-02	200 preps

Manual Date: September 2022
Revision Number: v5.0

For Research Use Only

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MicroElute®Cycle Pure Kit
MicroElute® Gel Extraction Kit

Table of Contents

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Introduction and Overview

The E.Z.N.A.® family of products is an innovative system that radically simplifies the extraction and purification of nucleic acids from a variety of sources. The key to this system is the HiBind® matrix that specifically, but reversibly, binds DNA or RNA under optimized conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or a low salt buffer.

The MicroElute® Clean Up system, designed for rapid DNA clean up, includes:

- MicroElute® Cycle-Pure Kit - for direct purification of double or single stranded PCR products (100 bp - 10 kb) from amplification reactions
- MicroElute® Gel Extraction Kit - for extraction of DNA fragments (70 bp - 20 kb) from standard, or low-melt agarose gels in TAE (Tris-acetate/EDTA) or TBE (Tris-borate/EDTA) buffer

Binding Capacity:
Each MicroElute® LE DNA Column can bind ~10 µg of DNA.

New in this Edition:

September 2022
- Column equilibration protocol is now required for more consistent results.

August 2019
- Binding Buffer (XP2) has been renamed XP2 Binding Buffer. This is a name change only. The formulation has not changed.
- SPW Wash Buffer has been renamed SPW Buffer. This is a name change only. The formulation has not changed.
- D6296 (all sizes) has been discontinued and is no longer available to purchase.

March 2018
- DP Buffer has been renamed CP Buffer. This is a name change only. The formulation has not changed.

November 2017
- VAC-08 Vacuum Manifold has been discontinued and is no longer available for purchase.

Kit Contents

Product	D6293-00	D6293-01	D6293-02
Purifications	5	50	200
MicroElute® LE DNA Columns	5	50	200
2 mL Collection Tubes	5	50	200
CP Buffer	5 mL	30 mL	120 mL
DNA Wash Buffer	2.5 mL	25 mL	3 x 25 mL
Elution Buffer	2 mL	30 mL	30 mL
User Manual	✓	✓	✓

Product	D6294-00	D6294-01	D6294-02
Purifications	5	50	200
MicroElute® LE DNA Columns	5	50	200
2 mL Collection Tubes	5	50	200
XP2 Binding Buffer	5 mL	30 mL	120 mL
SPW Buffer	2.5 mL	25 mL	3 x 25 mL
Elution Buffer	2 mL	30 mL	30 mL
User Manual	✓	✓	✓

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

Kit	100% Ethanol to be Added
D6293-00	10 mL
D6293-01	100 mL
D6293-02	100 mL per bottle

- Dilute SPW Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D6294-00	10 mL
D6294-01	100 mL
D6294-02	100 mL per bottle

Storage and Stability

All of the MicroElute® Kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in CP Buffer. Dissolve such deposits by warming the solution at 37°C and gently shaking.

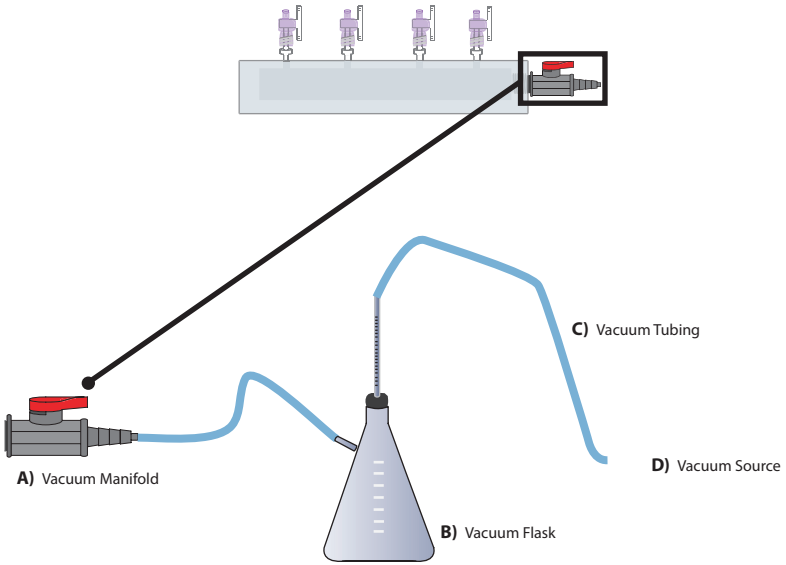
Guidelines for Vacuum Manifold

The following is required for use with the Vacuum/Spin Protocol:

A) Vacuum Manifold
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, Promega Vacman®, or manifold with standard Luer connector
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

Conversion from millibars:	Multiply by:
Millimeters of mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Vacuum Setup:



MicroElute® Cycle-Pure Kit - Centrifugation Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000*g*
- Vortexer
- 1.5 mL microcentrifuge tubes
- 100% ethanol
- 3M NaOH
- Sterile deionized water
- For fragments < 200 bp: 100% isopropanol

Before Starting:

- Prepare DNA Wash Buffer according to "Preparing Reagents" section on Page 4.

1. Perform agarose gel/ethidium bromide electrophoresis to analyze PCR product.

2. Determine the volume of your PCR reaction.

3. Transfer the sample into a clean 1.5 mL microcentrifuge tube.

4. Add 5 volumes CP Buffer. For fragments < 200 bp, add 5 volume CP Buffer and 0.4 volumes 100% isopropanol.

   Note: Volume refers to the size of your PCR reaction. For example, if your PCR reaction is 50 µL, you would use 250 µL CP Buffer. If fragments are less than 200 bp, then add 250 µL CP Buffer and 20 µL 100% isopropanol.

5. Vortex to mix thoroughly. Briefly centrifuge to collect any drops from the inside of the lid.

6. 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.

7. Transfer the sample from Step 5 to the MicroElute® LE DNA Column.

8. Centrifuge at maximum speed (≥13,000*g*) for 1 minute at room temperature.

9. Discard the filtrate and reuse collection tube.

10. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see the Preparing Reagents section on Page 4 for instructions.

11. Centrifuge at maximum speed for 1 minute.

12. Discard the filtrate and reuse collection tube.

13. Repeat Steps 10-12 for a second DNA Wash Buffer wash step.

14. 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**.**

15. Transfer the MicroElute® LE DNA Column into a clean 1.5 mL microcentrifuge tube (not provided).

16. Add 10-20 µL Elution Buffer, TE Buffer, or sterile deionized water directly to the center of column matrix.

17. Let sit at room temperature for 2 minutes.

18. Centrifuge at maximum speed for 1 minute.

    Note: This represents approximately 80-90% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

19. Store DNA at -20°C.

MicroElute® Cycle Pure Kit - Vacuum Protocol

Materials and Equipment to be Supplied by User:

- Vacuum Manifold
- Microcentrifuge capable of at least 13,000*g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- 3M NaOH
- Sterile deionized water
- For fragments < 200 bp: 100% isopropanol

Before Starting:

- Prepare DNA Wash Buffer according to the "Preparing Reagents" section on Page 4.

1. Perform agarose gel/ethidium bromide electrophoresis to analyze PCR product.

2. Determine the volume of your PCR reaction.

3. Transfer the sample into a clean 1.5 mL microcentrifuge tube.

4. Add 5 volumes CP Buffer. For fragments < 200 bp, add 5 volume CP Buffer and 0.4 volumes 100% isopropanol.

   Note: Volume refers to the size of your PCR reaction. For example, if your PCR reaction is 50 µL, you would use 250 µL CP Buffer. If fragments are less than 200 bp, then add 250 µL CP Buffer and 20 µL 100% isopropanol.

5. Vortex to mix thoroughly. Briefly centrifuge to collect any drops from the inside of the lid.

6. Prepare the vacuum manifold according to manufacturer's instructions. Connect the MicroElute® LE DNA Column to the manifold 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. Switch on vacuum source to draw the buffer through the column.
3. Turn off the vacuum.
4. Add 500 µL sterile deionized water to the MicroElute® LE DNA Column.
5. Switch on vacuum source to draw the water through the column.
6. Turn off the vacuum.

7. Transfer the entire sample from Step 5 to the MicroElute® LE DNA Column.

8. Switch on vacuum source to draw the sample through the column.

9. Turn off the vacuum.

10. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see the Preparing Reagents section on Page 4 for instructions.

11. Switch on vacuum source to draw the DNA Wash Buffer through the column.

12. Turn off the vacuum.

13. Repeat Steps 10-12 for a second DNA Wash Buffer wash step.

14. Transfer the MicroElute® LE DNA Column into a 2 mL Collection Tube (provided).

15. 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**.**

16. Transfer the MicroElute® LE DNA Column into a clean 1.5 mL microcentrifuge tube (not provided).

17. Add 10-20 µL Elution Buffer, TE Buffer, or sterile deionized water directly to the center of column matrix.

18. Let sit at room temperature for 2 minutes.

19. Centrifuge at maximum speed for 1 minute.

    Note: This represents approximately 80-90% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

20. Store DNA at -20°C.

MicroElute® Gel Extraction Kit - Centrifugation Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000*g*
- Incubator capable of 55°C
- Vortexer
- 1.5 mL microcentrifuge tubes
- 100% ethanol
- 3M NaOH
- Sterile deionized water

Before Starting:

- Prepare SPW Buffer according to "Preparing Reagents" section on Page 4.

1. Perform agarose gel/ethidium bromide electrophoresis to fractionate DNA fragments. Any type or grade of agarose may be used. However, it is strongly recommended that fresh TAE buffer or TBE buffer be used as running buffer. Do not reuse running buffer as its pH will increase and reduce yields.

2. When adequate separation of bands has occurred, carefully excise the DNA fragment of interest using a wide, clean, sharp scalpel. Minimize the size of the gel slice by removing extra agarose.

3. Determine the appropriate volume of the gel slice by weighing it in a clean 1.5 mL microcentrifuge tube. Assuming a density of 1 g/mL, the volume of gel is derived as follows: a gel slice of mass 0.3 g will have a volume of 0.3 mL.

4. Add 1 volume XP2 Binding Buffer.

5. Incubate at 60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes.

Important: Monitor the pH of the Gel/Binding Buffer mixture after the gel has completely dissolved. DNA yields will significantly decrease when the pH > 8.0. If the color of the mixture becomes orange or red, add 5 µL 5M sodium acetate (pH 5.2) to bring the pH down. After this adjustment, the color of the Gel/Binding Buffer mixture should be light yellow.

6. Insert a MicroElute® LE DNA Column in 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.

7. Transfer no more than 700 µL DNA/agarose solution from Step 5 to the MicroElute® LE DNA Column.

   Note: Each MicroElute® LE DNA Column has a total capacity of 10 µg DNA. If the expected yield is larger, divide the sample into the appropriate number of columns.

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

9. Discard the filtrate and reuse collection tube.

10. Repeat Steps 7-9 until all of the sample has been transferred to the column.

11. Add 300 µL XP2 Binding Buffer.

12. Centrifuge at maximum speed (≥13,000*g*) for 30 seconds at room temperature.

13. Discard the filtrate and reuse collection tube.

14. Add 700 µL SPW Buffer.

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

15. Centrifuge at maximum speed for 1 minute at room temperature.

16. Discard the filtrate and reuse collection tube.

Optional: Repeat Steps 14-16 for a second SPW Buffer wash step. Perform the second wash step for any salt sensitive downstream applications.

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

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

18. Transfer the MicroElute® LE DNA Column to a clean 1.5 mL microcentrifuge tube.

19. Add 10-20 µL Elution Buffer or sterile deionized water directly to the center of the column membrane.

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

20. Let sit at room temperature for 2 minutes.

21. 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.

22. Store DNA at -20°C.

MicroElute® Gel Extraction Kit - Vacuum Protocol

Materials and Equipment to be Supplied by User:

- Vacuum Manifold
- Microcentrifuge capable of at least 13,000*g*
- Incubator capable of 55°C
- Vortexer
- 1.5 mL microcentrifuge tubes
- 100% ethanol
- 3M NaOH
- Sterile deionized water

Before Starting:

- Prepare SPW Buffer according to "Preparing Reagents" section on page 4.

1. Perform agarose gel/ethidium bromide electrophoresis to fractionate DNA fragments. Any type or grade of agarose may be used. However, it is strongly recommended that fresh TAE buffer or TBE buffer be used as running buffer. Do not reuse running buffer as its pH will increase and reduce yields.

2. When adequate separation of bands has occurred, carefully excise the DNA fragment of interest using a wide, clean, sharp scalpel. Minimize the size of the gel slice by removing extra agarose.

3. Determine the appropriate volume of the gel slice by weighing it in a clean 1.5 mL microcentrifuge tube. Assuming a density of 1 g/mL, the volume of gel is derived as follows: a gel slice of mass 0.3 g will have a volume of 0.3 mL.

4. Add 1 volume XP2 Binding Buffer.

5. Incubate at 60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes.

Important: Monitor the pH of the Gel/Binding Buffer mixture after the gel has completely dissolved. DNA yields will significantly decrease when the pH > 8.0. If the color of the mixture becomes orange or red, add 5 µL 5M sodium acetate (pH 5.2) to bring the pH down. After this adjustment, the color of the Gel/Binding Buffer mixture should be light yellow.

6. Prepare the vacuum manifold according to manufacturer's instructions.

7. Connect the MicroElute® LE DNA Column to the vacuum manifold 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. Switch on vacuum source to draw the buffer through the column.
3. Turn off the vacuum.
4. Add 500 µL sterile deionized water to the MicroElute® LE DNA Column.
5. Switch on vacuum source to draw the water through the column.
6. Turn off the vacuum.

8. Add no more than 700 µL DNA/agarose solution from Step 5 to the MicroElute® LE DNA Column.

9. Turn on the vacuum source to draw the sample through the column.

10. Turn off the vacuum.

11. Repeat Steps 8-10 until all of the sample has been transferred to the column.

12. Add 300 µL XP2 Binding Buffer.

13. Turn on the vacuum source to draw the sample through the column.

14. Turn off the vacuum.

15. Add 700 µL SPW Buffer.

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

16. Turn on the vacuum source to draw the sample through the column.

17. Turn off the vacuum.

18. Repeat Steps 15-17 for a second SPW Buffer wash step.

19. Transfer the MicroElute® LE DNA Column to a clean 1.5 mL microcentrifuge tube.

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

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

21. Transfer the MicroElute® LE DNA Column to a clean 1.5 mL microcentrifuge tube.

22. Add 10-20 µL Elution Buffer or sterile deionized water directly to the center of the column membrane.

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

23. Let sit at room temperature for 2 minutes.

24. 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.

25. 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**.

Problem	Cause	Solution
Low DNA Yields	Not enough CP Buffer added to sample	Add more CP Buffer as indicated. For DNA fragments < 200 bp in size, add up to 6 volumes CP Buffer.
	pH of the sample mixture is too high	Add 10-20 µL sodium acetate (pH 5.2) to the sample and mix.
	Column Equilibration not performed	Perform the column equilibration protocol as instructed in the manual for consistent results.
Problem	Cause	Solution
Clogged Column in Gel Extraction	Incompletely dissolved gel	• Increase incubation time. • Increase XP2 Binding Buffer volume.
No DNA Eluted	SPW Buffer or DNA Wash Buffer was not diluted with ethanol	Prepare SPW Buffer or DNA Wash Buffer as instructed on Page 4.
Optical densities do not agree with DNA yield on agarose gel	Trace contaminants eluted from column increase A 260	Wash column as instructed. Alternatively, rely on agarose gel/ ethidium bromide electrophoresis for quantification.
DNA sample floats out of well while loading agarose gel	Ethanol not removed completely from column following wash steps	Centrifuge column as instructed to dry before proceeding to elution.

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

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