



E.Z.N.A.[®] Gel Extraction Kit

| | |
|----------|-----------|
| D2500-00 | 5 preps |
| D2500-01 | 100 preps |
| D2500-02 | 200 preps |

Manual Date: January 2019
Revision Number: v4.0

For Research Use Only

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E.Z.N.A.® Gel Extraction Kit

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Introduction

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.

Gel purification of DNA is a common technique for the isolation of specific fragments from reaction mixtures. However, most methods either fail to completely remove agarose or shear DNA which can lead to problems in downstream manipulations. The E.Z.N.A.® Gel Extraction Kit uses proprietary chemistry and HiBind® technology to recover DNA fragments between 70 bp and 20 kb with yields exceeding 85%. The DNA band of interest is excised from the gel, dissolved in Binding Buffer, and transferred to a HiBind® DNA Mini Column. Following three rapid wash steps, DNA is eluted with the Elution Buffer and is ready for other applications. DNA is suitable for ligations, PCR, sequencing, restriction digestion, or various labeling reactions. In addition, this kit can be also used to recover DNA directly from enzymatic reactions such as PCR and enzyme digestion reactions.

Benefits of the E.Z.N.A.® Gel Extraction Kit

- **Fast** - DNA recovery from agarose gel < 10 minutes
- **Reliability** - Optimized buffers that guarantee pure DNA
- **Safety** - No organic extractions
- **Quality** - Purified DNA is suitable for most applications

Binding Capacity

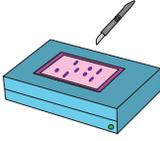
Each HiBind® DNA Mini Column can bind ~25 µg DNA.

New In this Edition

January 2019:

- SPW Wash Buffer has been renamed SPW Buffer and Binding Buffer (XP2) has been renamed XP2 Binding Buffer. These are name changes only. The formulations have not changed.
- All sizes of D2501 have been discontinued.

Spin Protocol



Excise DNA Band
from Gel



Incubate and Melt Gel
with Binding Buffer



Transfer Lysate & Bind



Wash 3x

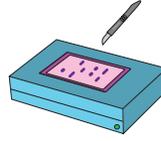


Dry



Elute

Vacuum/Spin Protocol



Excise DNA Band
from Gel



Incubate and Melt Gel
with Binding Buffer



Transfer Lysate & Bind



Wash 3x



Dry



Elute

Kit Contents and Storage

| Product | D2500-00 | D2500-01 | D2500-02 |
|--------------------------|----------|-----------|-----------|
| Purifications | 5 | 100 | 200 |
| HiBind® DNA Mini Columns | 5 | 100 | 200 |
| 2 mL Collection Tubes | 5 | 100 | 200 |
| XP2 Binding Buffer | 5 mL | 80 mL | 150 mL |
| SPW Buffer | 5 mL | 2 x 25 mL | 3 x 25 mL |
| Elution Buffer | 2 mL | 15 mL | 30 mL |
| User Manual | ✓ | ✓ | ✓ |

Storage and Stability

All E.Z.N.A.® Gel Extraction Kit components are guaranteed for at least 24 months from the date of purchase when stored at room temperature. Please ensure that the bottle of XP2 Binding Buffer is tightly capped when not in use. If any precipitates form in the buffers, warm at 37°C to dissolve.

Preparing Reagents

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

| Kit | 100% Ethanol to be Added |
|----------|--------------------------|
| D2500-00 | 20 mL |
| D2500-01 | 100 mL per bottle |
| D2500-02 | 100 mL per bottle |

Guideline for Vacuum Manifold

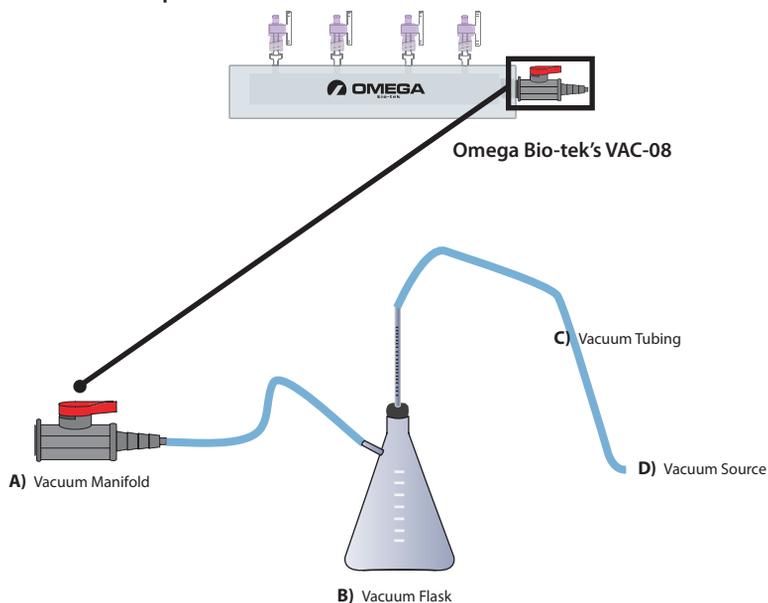
The following is required for use with the Vacuum/Spin Protocol:

- A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-08)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma Aldrich VM20, Promega Vacman®, or manifold with standard Luer connector
- B) Vacuum Flask
- C) Vacuum Tubing
- D) Vacuum Source (review tables below for pressure settings)

| Manifold | Recommended Pressure (mbar) |
|----------|-----------------------------|
| VAC-08 | -200 to -600 |

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

Illustrated Vacuum Setup:



E.Z.N.A.® Gel Extraction Kit - Spin Protocol

E.Z.N.A.® Gel Extraction Kit - Spin Protocol

Materials and Equipment to be Supplied by User:

- Heat block or water bath capable of 60°C
- Microcentrifuge capable of at least 13,000 x *g*
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- Optional: 5M Sodium Acetate, pH 5.2
- Optional: Sterile deionized water

Before starting:

- Prepare SPW Buffer according to the “Preparing Reagents” section on Page 5
- Set heating block or water bath to 50-60°C

Note: The yellow color of the XP2 Binding Buffer signifies a pH of ≤ 7.5 .

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 50-60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes.

E.Z.N.A.® Gel Extraction Kit - Spin Protocol

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 HiBind® DNA Mini Column in a 2 mL Collection Tube.
7. Add no more than 700 µL DNA/agarose solution from Step 5 to the HiBind® DNA Mini Column.
8. Centrifuge at 10,000 x *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 ($\geq 13,000 \times g$) for 1 minute 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 5 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.

E.Z.N.A.[®] Gel Extraction Kit - Spin Protocol

17. 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.

18. Transfer the HiBind[®] DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

19. Add 15-30 μ L Elution Buffer or 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 eluting DNA with 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.

E.Z.N.A.® Gel Extraction Kit - Vacuum Protocol

E.Z.N.A.® Gel Extraction Kit - Vacuum Protocol

Materials and Equipment to be Supplied by User:

- Vacuum Manifold (See Page 6)
- Heat block or water bath capable of 60°C
- Microcentrifuge capable of at least 13,000 x *g*
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- Optional: 5M Sodium Acetate, pH 5.2
- Optional: Sterile deionized water

Before starting:

- Prepare the Vacuum Manifold (See Page 6)
- Prepare SPW Buffer according to the "Preparing Reagents" section on Page 5
- Set heating block or water bath to 50-60°C

Note: The yellow color of the XP2 Binding Buffer signifies a pH of ≤ 7.5 .

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.

E.Z.N.A.[®] Gel Extraction Kit - Vacuum Protocol

5. Incubate at 50-60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes.
6. Prepare the vacuum manifold according to manufacturer's instructions.
7. Connect the HiBind[®] DNA Mini Column to the vacuum manifold.
8. Add no more than 700 µL DNA/agarose solution from Step 5 to the HiBind[®] DNA Mini 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 5 for instructions.
16. Turn on the vacuum source to draw the sample through the column.
17. Turn off the vacuum.

Optional: Repeat Steps 15-17 for a second SPW Buffer wash step. Perform the second wash step for any salt sensitive downstream applications.

E.Z.N.A.® Gel Extraction Kit - Vacuum Protocol

18. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.
19. 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.

20. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.
21. Add 15-30 μ L Elution Buffer or 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 eluting DNA with deionized water, make sure that the pH is around 8.5.

22. Let sit at room temperature for 2 minutes.
23. 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.

24. Store DNA at -20°C.

E.Z.N.A.® Gel Extraction Kit - Enzymatic Reaction Protocol

E.Z.N.A.® Gel Extraction Kit Protocol - Purification of DNA from Enzymatic Reactions

The following protocol is designed for DNA recovery from enzymatic reactions such as PCR and probe labeling reactions.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol

Before starting:

- Prepare SPW Buffer according to the "Preparing Reagents" section on Page 5

Note: The yellow color of the XP2 Binding Buffer signifies a pH of ≤ 7.5 .

1. Determine the volume of the enzymatic reaction.
2. Transfer the sample into a clean 1.5 mL microcentrifuge tube.
3. Add 1 volume XP2 Binding Buffer.
4. Vortex or invert the sample to mix thoroughly.
5. Briefly centrifuge the tube to collect any drops from the inside of the lid.
6. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.
7. Transfer the sample to the HiBind® DNA Mini Column.
8. Centrifuge at 10,000 x *g* for 1 minute at room temperature.

E.Z.N.A.® Gel Extraction Kit - Enzymatic Reaction Protocol

9. Discard the filtrate and reuse the collection tube.

10. Add 700 μ L SPW Buffer.

Note: SPW Buffer must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

11. Centrifuge at maximum speed ($\geq 13,000 \times g$) for 1 minute at room temperature.

12. Discard the filtrate and reuse collection tube.

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

14. 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.

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

16. Add 15-30 μ L Elution Buffer or 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 eluting DNA with deionized water, make sure that the pH is around 8.5.

17. Let sit at room temperature for 2 minutes.

18. 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.

19. 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 (800-832-8896).

Possible Problems and Suggestions

| Low DNA Yields | |
|--|---|
| Too little XP2 Binding Buffer added to gel | Volume of agarose gel slice determined incorrectly. Add enough XP2 Binding Buffer as instructed. |
| Agarose gel does not completely dissolve | Make sure water bath is set to 60°C and allow gel to completely melt. Add more XP2 Binding Buffer if necessary. |
| Inappropriate elution buffer | Use Elution Buffer or check the pH of the water used to elute DNA. |
| TAE/TBE running buffer is not fresh | With overuse, TAE buffer loses its buffering capacity and its pH increases. This raises the pH of the agarose/DNA Binding Buffer solution which interferes with DNA binding to the HiBind® matrix. Adjust pH by adding 5 µL 5M sodium acetate, pH 5.2, to the gel slice. Use freshly prepared TAE buffer for gel purification in order to prevent the contamination of isolated DNA and improve yields. |
| Column clogged | |
| Agarose gel not completely dissolved in XP2 Binding Buffer | Make sure water bath is set to 55-60°C and allow gel to completely melt. For large agarose slices (>0.3 mL), it is recommended that the gel be diced into smaller fragments to aid melting. |
| No DNA eluted | |
| SPW Buffer not diluted with 100% ethanol | Prepare SPW Buffer as instructed on page 5 or as indicated on bottle. |
| Incorrect amount of Binding Buffer added | Measure the gel accurately and use 0.1 mL XP2 Binding Buffer per 0.1 g gel. |
| Optical densities do not agree with DNA yield on agarose gel | |
| Trace contaminants eluted from column increase A_{260} | Make sure to wash column as instructed in Step 14 of the spin protocol, and Step 15 of the vacuum protocol. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantization. |
| DNA sample floats out of well while loading agarose gel. | |
| Ethanol not completely removed from column | Centrifuge as instructed in Step 17 of the spin protocol and Step 19 of the vacuum protocol. |

Ordering Information

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

| Product | Part Number |
|-----------------------------|-------------|
| XP2 Binding Buffer (200 mL) | PDR040 |
| XP2 Binding Buffer (500 mL) | PDR041 |
| SPW Buffer (25 mL) | PDR045 |
| 2 mL Collection Tubes | SS1-1370-00 |

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PCR is a patented process of Hoffman-La Roche. Use of the PCR process requires a license.

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NGS Clean Up



Tissue



FFPE



Fecal Matter



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