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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 new HiBind® matrix that specifically, but reversibly, binds DNA or RNA under certain optimal 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 cleanup, includes:

- **MicroElute™ Cycle-Pure Kits** for direct purification of double or single stranded PCR products (100 bp - 10 kb) from amplification reactions.
- **MicroElute™ Gel Extraction Kits** 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.
- **MicroElute™ DNA Cleanup Kits** for general cleanup of oligonucleotides and DNA up to 10 kb from enzymatic reactions (e.g., labeling, dephosphorylation, restriction, and tailing).

Benefits of the MicroElute™ DNA Cleanup System

- **Low elution volume**-Elute DNA with volume as low as 10µl
- **Reliability** -Optimized buffers that guarantee pure DNA every time
- **Safety**-No organic extractions
- **Quality**-Purified DNA will be suitable for most applications

Binding Capacity

Each **MicroElute™** HiBind® DNA column can bind ~ 10 µg DNA.

Kit Contents

Product Number	D6293-00	D6293-01	D6293-02
Purification Times	5 preps	50 preps	200 preps
MicroElute™ HiBind® DNA Columns	5	50	200
2 ml Collection Tubes	5	50	200
Buffer CP*	5 ml	30 ml	120 ml
Elution Buffer*	1 ml	15 ml	15 ml
DNA Wash Buffer	1.5 ml	15 ml	3 x25 ml
Instruction Booklet	1	1	1

*Buffer CP contains chaotropic salts which are irritants. Take appropriate laboratory safety measures and wear gloves when handling.

* Elution Buffer = 10mM Tris-HCl, pH 8.5

Storage and Stability

All MicroElute™ Cycle-Pure Kit components are guaranteed for at least 24 months from the date of purchase when stored at 15-25°C. Under cool ambient conditions crystals may form in Buffer CP. Simply warm to 37°C to dissolve.

Before Starting

It is strongly advised that you familiarize yourself with the entire procedure before beginning this protocol. The MicroElute™ DNA Kit is designed to be simple, fast, and reliable provided that all steps are followed diligently.

Important	Dilute DNA Wash Buffer with absolute ethanol (96-100%) as follows	
	D6293-00	Add 6 ml of absolute ethanol
	D6293-01	Add 60 ml of absolute ethanol
	D6293-02	Add 100 ml absolute ethanol to each bottle

MicroElute™ Cycle-Pure Spin Protocol

Materials Supplied by User

- Microcentrifuge capable of at least 13,000 x g
- (Optional) Sterile deionized water (or TE Buffer)
- Absolute ethanol (~ 96-100%)

Please Note:

- All centrifugation steps must be performed at room temperature
- DNA Wash Buffer must be diluted with absolute ethanol prior to use (see previous section, or label instructions)

1. **Perform agarose gel/ethidium bromide electrophoresis to analyze PCR product.**
2. **Determine the volume of the PCR reaction, transfer sample into a clean 1.5 ml microcentrifuge tube, and add 5 volumes of Buffer CP.** For example, add 250 µl of Buffer CP to 50 µl PCR sample.
3. **Vortex thoroughly to mix. Briefly spin the tube to collect any drops from the inside of the lid.**
4. **Place a MicroElute™ HiBind® DNA Column in a provided 2 ml collection tube.**
5. **Apply the sample to the MicroElute™ HiBind® DNA Column, and centrifuge at 10,000 x g for 1 min at room temperature.**
6. **Discard liquid and place the MicroElute™ HiBind® DNA Column back into the same collection tube.**
7. **Wash the MicroElute™ HiBind® DNA Column by adding 700 µl of DNA Wash Buffer diluted with absolute ethanol. Centrifuge at 10,000 x g for 1 min at room temperature.**

NOTE: DNA Wash Buffer must be diluted with absolute ethanol before use. See label for directions. If refrigerated, DNA Wash Buffer must be brought to room temperature before use.
8. **Discard liquid and repeat step 7 with another 700 µl of DNA Wash Buffer.**
9. **Discard liquid and centrifuge the empty MicroElute™ HiBind® column for 2 min**

at maximum speed ($\geq 13,000 \times g$) to dry the column matrix. *This is critical for good yields.*

10. Place MicroElute™ HiBind®DNA column into a clean 1.5 ml microcentrifuge tube. Add 10-20 μ l (depending on desired concentration of final product) of Elution Buffer (10mM Tris, pH8.5) or water directly onto the column matrix and let it sit at room temperature for 1-2 minutes. Centrifuge for 1 min at $\geq 13,000 \times g$ to elute DNA. This represents approximately 80-90% of bound DNA. An OPTIONAL second elution will yield any residual DNA, though at a lower concentration.

11. Yield and quality of DNA: Determine the absorbance of an appropriate dilution of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

$$\text{DNA concentration} = A_{260} \times 50 \times (\text{Dilution Factor}) \mu\text{g/ml}$$

Fragments greater than 500 bp in length can routinely be purified at > 80% yield. Bands ranging from 100 bp to 500bp gives yields of 60%-90%. The ratio of (A_{260}) / (A_{280}) is an indication of nucleic acid purity. Alternatively, yield (as well as quality) can sometimes be best determined by agarose gel/ethidium bromide electrophoresis.

MicroElute™ Cycle-Pure Vacuum/Spin Protocol

Please read through previous sections of this book before using this protocol.

1. Prepare the sample by following steps 1-3 of the spin protocol on page 4.
2. Prepare the vacuum manifold according to manufacturer's instructions.
3. Load the PCR reaction/CP solution from step 3 (of spin protocol) to the MicroElute™ HiBind® DNA column by decanting or pipetting, and apply vacuum. After the samples have passed through the column, switch off the vacuum source.

HiBind® DNA Column by adding 700 μ l of DNA Wash Buffer diluted with absolute ethanol and turning on the vacuum source. Repeat this step with another 700 μ l of DNA Wash Buffer.

5. Assemble the MicroElute™ HiBind® DNA Column into a 2 ml collection tube. Spin for 2 min at maximum speed ($\geq 13,000 \times g$) to dry the MicroElute™ HiBind®DNA column.
4. Wash the MicroElute™

6. Place the MicroElute™ HiBind® DNA Column in a clean 1.5 ml microcentrifuge tube and add 10 - 20 μ l of Elution Buffer. Let it sit at room temperature for 1-2 minutes. Centrifuge for 1 min at $\geq 13,000 \times g$ to elute DNA.

MicroElute™ Gel Extraction Kit

Kit Contents

Product Number	D6294-00	D6294-01	D6294-02
Purification Times	5 preps	50 preps	200 preps
MicroElute™ HiBind®DNA Columns	5	50	200
2 ml Collection Tubes	5	50	200
Binding Buffer* (XP2)	5 ml	30 ml	120 ml
Elution Buffer*	1 ml	15 ml	15 ml
SPW Wash Buffer	2 ml	25 ml	3 x 25 ml
Instruction Booklet	1	1	1

*Binding Buffer contains chaotropic salts which are irritants. Take appropriate laboratory safety measures and wear gloves when handling.

* Elution Buffer = 10mM Tris-HCl, pH 8.5

Storage and Stability

All MicroElute™ Gel Extraction Kit components are guaranteed for at least 24 months from the date of purchase when stored at 15-25°C. Please ensure that the bottle of Binding Buffer is tightly capped when not in use.

Before Starting

It is strongly advised that you familiarize yourself with the entire procedure before beginning this protocol.

Important	Dilute SPW Wash Buffer with absolute ethanol (96-100%) as follows	
	D6294-00	Add 8 ml of absolute ethanol to bottle
	D6294-01	Add 100 ml of absolute ethanol to bottle
	D6294-02	Add 100 ml absolute ethanol to each bottle

MicroElute™ Gel Extraction Spin Protocol

Materials Supplied by User

- Microcentrifuge capable of at least 13,000 x g
- (Optional) Sterile deionized water (or TE Buffer)
- Absolute ethanol (~ 96-100%)
- Protective eye-ware

1. **Perform agarose gel/ethidium bromide electrophoresis to fractionate DNA fragments.** Any type or grade of agarose may be used. It is strongly recommended however, 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 UV light box ensuring that as much agarose gel as possible has been removed.** Avoid more than 30 seconds of exposure of UV light to the DNA. Always use protective eye-ware when working with UV light.
3. **Determine the appropriate volume of the gel slice by weighing it in a clean 1.5 ml microcentrifuge tube.** Assuming a density of 1g/ml of gel, the volume of gel is derived as follows: a gel slice of mass 0.2g will have a volume of 0.2 ml. **Add an equal volume of Binding Buffer. Incubate the mixture at 55-60 °C for 7 min or until the gel has completely melted. Mix by shaking or vortexing the tube in increments of 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 pH > 8.0. If the color of the mixture becomes orange or red, add 5 µl of 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.

4. **Place a MicroElute™ HiBind® DNA Column in a provided 2 ml collection tube.**
5. **Apply the DNA/agarose solution (no more than 700 µl at a time) to the MicroElute™ HiBind® DNA Column, and centrifuge at 10,000 x g for 1 min at room temperature.**
6. **Discard liquid and place the MicroElute™ HiBind® DNA Column back into the same collection tube.** For volumes greater than 700 µl, load the column and centrifuge successively, 700 µl at a time. Each MicroElute™ HiBind® 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.

7. **Add 300 µl of Binding Buffer (XP2) into the MicroElute™ HiBind® DNA Column. Centrifuge as above to wash the column.**
8. **Discard the flow-through and wash the MicroElute™ HiBind® DNA Column by adding 700 µl of SPW Wash Buffer diluted with absolute ethanol. Centrifuge as above and discard the flow-through.**

Note: SPW Wash Buffer must be diluted with absolute ethanol before use. See label for directions.
9. **OPTIONAL: Repeat step 8 with another 700 µl of SPW Wash Buffer.**
10. **Discard liquid and centrifuge the empty MicroElute™ HiBind® DNA Column for 2 min at maxi speed (≥ 13,000 x g) to dry the column matrix.** This is critical for good yields.
11. **Place MicroElute™ HiBind® DNA Column into a clean 1.5 ml microcentrifuge tube. Add 10-20 µl (depending on desired concentration of final product) of Elution Buffer (10mM Tris, pH8.5) or water directly onto the column matrix and let it sit at room temperature for 1-2 minutes. Centrifuge for 1 min at ≥ 13,000 x g to elute DNA.** This represents approximately 70-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

MicroElute™ Gel Extraction Vacuum/Spin Protocol

1. Prepare the DNA/agarose solution by following steps 1-3 on page 7.
2. Prepare the vacuum manifold according to manufacturer's instructions.
3. **Load the DNA/agarose solution from step 3 to the MicroElute™ HiBind® DNA Column by decanting or pipetting, and apply vacuum.** After the samples have passed through the column, **switch off the vacuum source.**
4. **Wash the MicroElute™ HiBind® DNA Column by adding 300 µl of Binding Buffer (XP2) and turning on the vacuum source.**
5. **Wash the column again by adding 700 µl of SPW Wash Buffer diluted with absolute ethanol.** An **OPTIONAL** wash step may be performed with another 700 µl of SPW Wash Buffer.
6. **Assemble the MicroElute™ HiBind® DNA Column into a 2 ml collection tube. Spin for 2 min at maximal speed (≥ 13,000 x g) to dry the column.**

7. Place the **MicroElute™ HiBind® DNA Column** in a clean 1.5 ml microcentrifuge tube and add 10-20 µl of **Elution Buffer** (10mM Tris, pH8.5). Let it sit at room temperature for 1-2 minutes. Centrifuge for 1 min at $\geq 13,000 \times g$ to elute DNA.

MicroElute™ DNA Clean-Up Kit

Kit Contents

Product Number	D6296-00	D6296-01	D6296-02
Purification Times	5 preps	50 preps	200 preps
MicroElute™ HiBind® DNA Columns	5	50	200
2 ml Collection Tubes	5	50	200
Buffer DP	2 ml	20 ml	80 ml
Elution Buffer*	1 ml	15 ml	15 ml
SPW Wash Buffer	5 ml	25 ml	3 x 25 ml
Instruction Booklet	1	1	1

* Elution Buffer = 10mM Tris-HCl, pH 8.5

Storage and Stability

All MicroElute™ DNAClean-Up Kit components are guaranteed for at least 24 months from the date of purchase when stored at 15-25°C. Please ensure that the bottle of Buffer DP is tightly capped when not in use.

Before Starting

It is strongly advised that you familiarize yourself with the entire procedure before beginning this protocol.

Important	Dilute SPW Wash Buffer with absolute ethanol (96-100%) as follows	
	D6296-00	Add 20 ml of absolute ethanol
	D6296-01	Add 100 ml of absolute ethanol
	D6296-02	Add 100 ml absolute ethanol to each bottle

MicroElute™ DNA Clean-Up Spin Protocol

Materials Supplied by User

- (Optional) Sterile deionized water (or TE Buffer)
- Absolute ethanol (~ 96-100%)
- Microcentrifuge capable of at least 13,000 x g

1. **Determine the volume of the enzymatic reaction, transfer sample into a clean 1.5 ml microcentrifuge tube, and add 3 volumes of Buffer DP.** For example, add 300 µl of Buffer DP to 100 µl enzymatic reaction.
2. **Place a MicroElute™ HiBind® DNA Column in a provided 2 ml collection tube.**
3. **Apply the sample to the MicroElute™ HiBind® DNA Column, and centrifuge at 10,000 x g for 1 min at room temperature to bind DNA.**
4. **Discard liquid and place the MicroElute™ HiBind® DNA Column back into the same collection tube.**
5. **Wash the MicroElute™ HiBind® DNA Column by adding 700 µl of SPW Wash Buffer diluted with absolute ethanol and centrifuge as above.** Discard liquid and re-use the collection tube.

NOTE: SPW Wash Buffer must be diluted with absolute ethanol before use. See label for directions. If refrigerated, SPW Wash Buffer must be brought to room temperature before use.
6. **OPTIONAL: Repeat step 5 with another 700 µl of SPW Wash Buffer.**
7. **Discard liquid and place the MicroElute™ HiBind® DNA Column back into the same collection tube.**
8. **Centrifuge the empty MicroElute™ HiBind® DNA Column for 2 min at maximum speed ($\geq 13,000 \times g$) to dry the column matrix.** This is critical for good yields.
9. **Place MicroElute™ HiBind® DNA column into a clean 1.5 ml microcentrifuge tube. Add 10-20 µl of Elution Buffer (10mM Tris, pH8.5) directly onto the column matrix and incubate at room temperature for 1 min.**
10. **Centrifuge for 1 min at $\geq 13,000 \times g$ to elute DNA.** This represents approximately 80-90% of bound DNA. An **OPTIONAL** second elution will yield any residual DNA, though

at a lower concentration.

- Yield and quality of DNA: Determine the absorbance of an appropriate dilution of the sample at 260 nm and then at 280 nm.** The DNA concentration is calculated as: $\text{DNA concentration} = A_{260} \times 50 \times (\text{Dilution Factor}) \mu\text{g/ml}$.

Fragments greater than 500 bp in length can routinely be purified at 80% yield. Bands ranging from 100 bp to 500bp gives yields of 55-80%. The ratio of $(A_{260}) / (A_{280})$ is an indication of nucleic acid purity. A value greater than 1.8 indicates > 90% nucleic acid purity. Alternatively, yield (as well as quality) can sometimes be best determined by agarose gel/ethidium bromide electrophoresis.

MicroElute™ DNA Cleanup Vacuum/Spin Protocol

- Determine the volume of the enzymatic reaction, transfer sample into a clean 1.5 ml microcentrifuge tube, and add 3 volumes of Buffer DP.** For example, add 300 µl of Buffer DP to 100 µl enzymatic reaction.
- Prepare the vacuum manifold according to manufacturer's instructions, and **connect the V-Spin column to the manifold.**
- Load the sample to the MicroElute™ HiBind® DNA Column by decanting or pipetting, and apply vacuum. After the samples have passed through the column, switch off the vacuum source.**
- Wash the MicroElute™ HiBind® DNA Column by adding 700 µl of SPW Wash Buffer diluted with absolute ethanol and turning on the vacuum source.** An **OPTIONAL** wash step maybe performed with another 700 µl of SPW Wash Buffer.
- Assemble the MicroElute™ HiBind® DNA Column into a 2 ml collection tube. Spin for 2 min at maxi speed ($\geq 13,000 \times g$) to dry the MicroElute™ HiBind® column.**
- Place the MicroElute™ HiBind® DNA Column in a clean 1.5 ml microcentrifuge tube and add 10 - 20 µl of Elution Buffer (10mM Tris, pH 8.5). Let it sit at room temperature for 1-2 minutes. Centrifuge for 1 min at $\geq 13,000 \times g$ to elute DNA.**

Troubleshooting Guide

Causes	Solution
Low DNA yields	
Too Little Buffer CP, Binding Buffer or DP added to sample.	Add more buffer as indicated. For fragments <200 bp in size, add up to 6 x volume.
pH of the sample mixture is too high.	Add 10-20 µl of Sodium Acetate pH 5.2 to to the sample and mix.
Clogged Column in Gel Extraction	
Incompletely dissolved Gel.	Increase incubation time. Increase Binding Buffer Volume.
No DNA Eluted	
SPW or DNA Wash Buffer not diluted w ith ethanol.	Prepare SPW or DNA Wash Buffer Concentrate as instructed.
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 quantization.
DNA sample floats out of well while loading agarose gel.	
Ethanol not removed completely from column follow ing w ash steps.	Centrifuge column as instructed to dry before proceeding to elution.

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