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Introduction

The E.Z.N.A.™ family of products is an innovative system that radically simplifies extraction and purification of nucleic acids from a variety of sources. Key to the 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 low salt buffer.

The MicroElute™ DNA Clean-up system is a convenient system for fast and reliable purification of DNA from agarose gels, PCR reactions or enzyme reactions (such as labeling reaction) with relative small elution volume of 10-15µl. MicroElute™ DNA Clean-up system consist of 3 kits: MicroElute™ Gel Extraction Kit, MicroElute™ Cycle-Pure Kit and MicroElute™DNA Cleanup Kit.

The method uses HiBind® technology to recover DNA from agarose, PCR Reaction, and enzymatic reactions in yields exceeding 80%. Binding conditions are adjusted by addition of a specially formulated buffer, and the sample is applied to a HiBind® DNA spin-column. Following a rapid wash step, DNA is eluted with Elution Buffer or deionized water and ready for all downstream applications. No organic extractions or alcohol precipitations means safe and rapid processing of multiple samples in parallel. The product is suitable for T-A ligations, PCR sequencing, restriction digestion, or various labeling reactions.

Benefits

The MicroElute™ DNA System means:

- **Low elution volume** - Special designed column allows purification of concentrated DNA fragment in as little as 10µl.
- Speed - DNA recovery from enzymatic reactions <15 min
- Reliability - optimized buffers guarantee pure DNA
- Safety - No organic extractions
- Quality - purified DNA suitable for any application

Binding Capacity

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

MicroElute™ Cycle-Pure Kit

Product Number	D6293-00	D6293-01	D6293-02
Purification Times	5 Preps	50 Preps	200 Preps
HiBind® MicroElute™ 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 Concentrate	2 mL	20 mL	3 x 20 mL
Instruction Booklet	1	1	1

*Buffer CP contains chaotropic salts which are irritant. Ware gloves and other appropriate laboratory safety measures when handling.

Storage and Stability

All MicroElute™Cycle-Pure Kit components are guaranteed for at least 24 months from the date of purchase when stored at 22-25°C. Under cool ambient conditions crystals may form in Buffer CP. Simply warm to 37°C to dissolve.

Materials Supplied By User

- Microcentrifuge capable of at least 10,000 x g.
- Sterile deionized water (or TE buffer)
- Absolute (~96-100%) ethanol
- Protective eye-ware

IMPORTANT	**DNA Wash Buffer concentrate** must be diluted with absolute ethanol (~96-100%) as follows and store at room temperature.
	D6293-00 Add 8 mL absolute ethanol
	D6293-01 Add 80 mL absolute ethanol
	D6293-02 Add 80 mL absolute ethanol to each bottle

MicroElute™ Cycle-Pure Spin Protocol

Please read this booklet thoroughly to ensure that you are familiar with the entire procedure. MicroElute™ Cycle-Pure Kit is designed to be simple, fast, and reliable provided that all steps are followed diligently. All centrifugation steps must be performed at room temperature.

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

2. **Determine the volume of the PCR reaction, transfer to a clean 1.5 mL microcentrifuge tube, and add five volumes of Buffer CP**. For example, for 50 µl PCR reaction, add 250 µl of Buffer CP. Vortex thoroughly to mix. Briefly spin the tube to collect any drops from the inside of the lid.

3. **Apply the sample to a HiBind® MicroElution™ DNA column assembled in a clean 2 mL collection tube (provided) and centrifuge at 10,000 x g for 1 min at room temperature.** Discard the liquid and re-use the collection tube.

4. **Wash the 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 Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for directions.

5. **Discard liquid and repeat step 4 with another 700 µl DNA Wash Buffer.**

6. **Discard liquid and centrifuge the empty column for 1 min at maximal speed (≥13,000 x g) to dry the column matrix. This is critical for good DNA yields.**

7. **Place column into a clean 1.5 mL microcentrifuge tube. Add 10-20 µl (depending on desired concentration of final product) Elution Buffer(10mM Tris, pH8.5) directly onto the column matrix and centrifuge for 1 min at 10,000 x 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.

8. **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:

   DNA concentration = Absorbance$_{260}$ × 50 × (Dilution Factor) µg/mL

   Fragments greater than 500 bp in length can routinely be purified at >80% yield. Bands ranging from 50 bp to 500 bp give yields of 60%-90%. The ratio of (absorbance$_{260}$)/(absorbance$_{280}$) is an indication of nucleic acid purity. A value greater than 1.8 indicates > 90% nucleic acid. Alternatively, yield (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis.

MicroElute™ Cycle-Pure Kit Vacuum/Spin Protocol

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

1. **Prepare the sample by following the Spin Protocol step 1-2 on page 4.**
2. Prepare the vacuum manifold according to manufacturer' s instruction and connect the V-Spin column to the manifold.
3. Load the PCR reaction/CP solution from step 2 to the column.
4. Switch on vacuum source to draw the sample through the column and turn off the vacuum.
5. Wash the column by adding 700 µl DNA Wash buffer, draw the DNA Wash Buffer through the column by turn on the vacuum source. Repeat this step with another 700 µl DNA Wash Buffer.
6. **Assemble the column into a 2 mL collection tube and transfer the column to a micro centrifuge. Spin at maximal speed (≥13,000 x g) for 1 minute to dry the column.**
7. Place the column in a clean 1.5 mL microcentrifuge tube and add 10-20µl Elution Buffer (10mM Tris, pH8.5). Stand for 1-2 minute and centrifuge at maximal speed (≥13,000 x g) for 1 minute at 10,000 x g to elute DNA.

MicroElute™ Gel Extraction Kit

Product Number	D6294-00	D6294-01	D6294-02
Purification Times	5 Preps	50 Preps	200 Preps
HiBind® MicroElute™ 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 Concentrate	2 mL	20 mL	3 x 20 mL
Instruction Booklet	1	1	1

*Binding Buffer contains chaotropic salts which are irritant. Ware gloves and other appropriate laboratory safety measures when handling.

Storage and Stability

All MicroElute™ Gel Extraction Kit components are guaranteed for at least to 24 months from the date of purchase when stored at 22-25°C. Ensure that the bottle of Binding Buffer is capped tightly when not in use.

Materials Supplied By User

- Sterile deionized water (or TE buffer)
- Absolute (or 95%) ethanol
- Protective eye-ware

IMPORTANT	**SPW Wash Buffer Concentrate** must be diluted with absolute ethanol (~96-100%) as follows D6294-00 Add 8 mL absolute ethanol D6294-01 Add 80 mL absolute ethanol D6294-02 Add 80 mL absolute ethanol /bottle

MicroElute™ Gel Extraction Spin Protocol

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 exposure of UV light to the DNA. *Always use protective eye-ware when working with UV light.***

3. **Determine the approximate volume of the gel slice by weighing it in a clean 1.5 mL microcentrifuge tube**. Assuming a density of 1 g/mL of gel, the volume of gel is derived as follows: a gel slice of mass 0.2 g will have a volume of 0.2 mL. **Add equal volume of Binding Buffer. Incubate the mixture at 55°C-60°C for 7 min or until the gel has completely melted.** Mix by shaking or vortexing the tube in every 2-3 minutes.

   Important: Monitor the pH of the Gel/Binding buffer mixture after the gel completely dissolved. DNA yield will significantly decreased when pH > 8.0. If the color of the mixture become 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. **Apply 700 µl of the DNA/agarose solution to a HiBind® MicroElute™ DNA column assembled in a clean 2 mL collection tube (provided) and centrifuge at 8000-10,000 x g for 1 min at room temperature.** Discard the liquid and re-use the collection tube. For volumes greater than 700 µl load the column and centrifuge successively, 700 µl at a time. Each HiBind® extraction column has a total capacity of 10-15 µg DNA. If the expected yield is larger, divide the sample into the appropriate number of columns.

5. **Add 300 µl of Binding Buffer (XP2) into the column.** Centrifuge at 10,000 x g for 1 min at room temperature to wash the column. Discard the flow-through and re-use the collection tube.

6. **Add with 700 µl SPW Wash Buffer diluted with absolute ethanol into the column and centrifuge at ≥10,000 x g for 1 minute at room temperature. Repeat this step with another 700 µl SPW Wash Buffer.**

   Note: SPW Wash Buffer Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for directions.

7. **Discard liquid and centrifuge the empty column for 1 min at maximal speed (≥13,000 x g) to dry the column matrix. This is critical for good DNA yields.**

8. **Place column into a clean 1.5 mL microcentrifuge tube. Add 10-20 µl (depending on desired concentration of final product) Elution Buffer (10mM Tris, pH8.5) or water directly onto the column matrix and centrifuge for 1 min at maximal speed (≥13,000 x g) to elute DNA.** This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

   Note: efficiency of eluting DNA from column is dependent on pH. If eluting DNA with water, make sure that the pH is around 8.0.

   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:

   DNA concentration = Absorbance$_{260}$ × 50 × (Dilution Factor) µg/mL

   Fragments greater than 500 bp in length can routinely be purified at >80% yield. Bands ranging from 50 bp to 500 bp give yields of 55%-80%. The ratio of (absorbance$_{260}$)/(absorbance$_{280}$) is an indication of nucleic acid purity. A value greater than 1.8 indicates > 90% nucleic acid. Alternatively, yield (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis.

MicroElute™ Gel Extraction Vacuum/Spin Protocol

Note: Please read through previous section before using this protocol.

1. **Prepare the gel sample and dissolve the gel by following the Gel Extraction Spin Protocol step 1-3 on page 7.**

2. Prepare the vacuum manifold according to manufacturer' s instruction and connect the V-Spin column to the manifold.

3. Load the dissolved DNA/agarose solution from step 3 to the column.

4. Switch on vacuum source to draw the sample through the column and turn off the vacuum.

5. Wash the column by adding 300 µl Binding Buffer, draw the Binding Buffer through the column by turn on the vacuum source.

6. Wash the column by adding 700 µl SPW Wash Buffer, draw the SPW Wash buffer through the column by turn on the vacuum source.

7. **Assemble the column into a 2 mL collection tube and transfer the column to a micro centrifuge. Spin at maximal speed (≥13,000 x g) for 1 minute to dry the column.**

8. Place the column in a clean 1.5 mL microcentrifuge tube and add 10-20µl Elution Buffer (10mM Tris, pH8.5). Stand for 1-2 minute and centrifuge at maximal speed (≥13,000 x g) for 1 minute to elute DNA

MicroElute™ DNA Clean-Up Kit

Product Number	D6296-00	D6296-01	D6296-02
Purification Times	5 Preps	50 Preps	200 Preps
HiBind™ MicroElute™ DNA Column	5	50	200
2mL Collection Tubes	5	50	200
Buffer P3	2 mL	40 mL	200mL
SPW Wash Buffer Concentrate	5 mL	20 mL	3 x 20 mL
Elution Buffer	1 mL	15 mL	15 mL
Instruction Booklet	1	1	1

Storage and Stability

All MicroElute™ DNA Clean-Up Kit components are guaranteed for at least to 24 months from the date of purchase when stored at 22-25°C. Ensure that the bottle of Buffer DP is capped tightly when not in use.

Materials Supplied By User

- Sterile 1.5 mL centrifuge tubes.
- Sterile deionized water (or TE buffer)
- Absolute (or 95%) ethanol
- Protective eye-ware

IMPORTANT	**SPW Wash Buffer Concentrate** must be diluted with absolute (~96-100%) ethanol as follows: D6296-00 Add 20 mL absolute ethanol D6296-01 Add 80 mL absolute ethanol D6296-02 Add 80 mL absolute ethanol/bottle

MicroElute™ DNA Clean-Up Spin Protocol

For DNA fragments (<10 kb)

1. **Add 5 x volume of buffer P3 to the enzymatic reaction and mix throughly with pipetting or vortex.** The maximum volume of the reaction can be processed per column is 100 µl. For example, 100 µl of enzymatic reaction, add 500 µl of Buffer P3. Proceed step 2.

For Genomic DNA Clean Up (>10 kb)

1. Add 0.5 x volume of Buffer P3 and 1 x volume of absolute ethanol (room temperature, 96-100%) to the DNA solution and vortex to mix well. **For example, 100 µl of DNA Solution, add 50 µl of Buffer P3 and 100 µl of absolute ethanol. Proceed step 2.**

   Tips: 1 volume of Buffer P3 and 2 volume of absolute ethanol can be premixed. Then add 1.5 x volume of this mixture into DNA solution.

Purify DNA by HiBind™ MicroElute™ DNA Column

2. Place a MicroElute HiBind™ DNA column into a 2 mL collection tube (supplied) in a tube rack.

3. **Apply all the sample mixture into the spin column with a micro pipettor.**

4. Spin at 10,000 x g for 1 minute to bind DNA.

5. Discard the flow-through and reuse the collection tubes for next step.

6. **Add 700 µl of SPW Wash buffer diluted with ethanol to the HiBind™ MicroElute™ DNA column and spin at 10,000 x g for 1 minute.** Discard the flow-through and place the column back into same 2mL collection tube.

7. Repeat Step 6 with another 700 µl of SPW Wash buffer diluted with ethanol.

8. Centrifuge at maximum speed (≥13,000 x g) for 2 minute to dry the column. Discard the 2 mL collection tubes.

9. Place the MicroElute™ HiBind™ DNA column into a clean 1.5 mL micro centrifuge tube.

10. **Add 15-20 µl Elution Buffer(10mM Tris, pH 8.5) or water to the center of the membrane, incubate at room temperature for 1 minute.**

11. Centrifuge at maximal speed (≥13,000 x g) for 1 minute to elute DNA.

12. **For genomic DNA clean up, repeat elution step 10-11 by adding another 15-20 µl Elution Buffer.**

MicroElute™ DNA Clean-Up Vacuum/Spin Protocol

Note: Please read through previous section before using this protocol.

1. Add 300 µl volume of DP buffer to the enzymatic reaction and mix throughly with pipetting or vortex. The maximum volume of the reaction can be processed per column is 100 µl.

2. Prepare the vacuum manifold according to manufacturer' s instruction and connect the V-Spin column to the manifold.

3. Load the dissolved DNA/agarose solution from step 3 to the column.

4. Switch on vacuum source to draw the sample through the column and turn off the vacuum.

5. Wash the column by adding 700 µl SPW Wash Buffer, draw the SPW Wash Buffer through the column by turn on the vacuum source.

6. **Assemble the column into a 2 mL collection tube and transfer the column to a micro centrifuge. Spin at maximal speed (≥ 13,000 x g) for1 minute to dry the column.**

7. Place the column in a clean 1.5 mL microcentrifuge tube and add 10-20µl Elution Buffer (10mM Tris, pH8.5). Stand for 1-2 minute and centrifuge at maximal speed (≥ 13,000 x g) for 1 minute to elute DNA.

Troubleshooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Too little Buffer CP, Binding buffer (XP2) or DP added to sample.	Add more Buffer CP, P3 or Binding Buffer as indicated. For DNA fragments <200 bp in size, add up to 6 x vol.
	PH of the sample mixture is too high	Add 10-20 µl Sodium Acetate, pH 5.2 to the sample and mix.
Clogged Column in Gel extraction	Gel dissolved incompletely	Increase Incubation time Increase Binding buffer volume
No DNA eluted.	SPW Buffer or DNA Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare SPW Buffer or DNA Wash Buffer Concentrate as instructed above.
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. 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 following wash steps.	Centrifuge column as instructed in step 7 to dry before proceeding to elution step.