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## Introduction

The E.Z.N.A.<sup>®</sup> 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<sup>®</sup> 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 Micro Spin<sup>™</sup> 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 10-15 $\mu$ l. Micro Spin<sup>™</sup> DNA Clean-up system consist of 3 kits: Micro Spin<sup>™</sup> Gel Extraction Kit, Micro Spin<sup>™</sup> Cycle-Pure Kit and Micro Spin<sup>™</sup> DNA Cleanup Kit.

The method uses HiBind<sup>®</sup> technology to recover DNA bands 60 bp-40 kb DNA free of agarose, oligonucleotides, nucleotides, and enzymes in yields exceeding 80%. Binding conditions are adjusted by addition of a specially formulated buffer, and the sample is applied to a HiBind<sup>®</sup> DNA spin-column. Following a rapid wash step, DNA is eluted with deionized water (or low salt buffer) and ready for other 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. In addition the kit can be used to purify DNA from any other enzymatic reaction.

### Benefits

The Micro Spin<sup>™</sup> DNA System means:

- **Low elution volume** - Special designed column allows purification of concentrated DNA fragment in as little as 10 $\mu$ l.
- **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 Micro Spin HiBind<sup>®</sup> DNA column can bind ~10  $\mu$ g DNA.

# The Micro Spin™ Cycle-Pure Kit

## Kit Contents

Product Number	D6293-00	D6293-01	D6293-02
Purification	5	50	100
Micro Spin™ HiBind® DNA Columns	5	50	100
2 ml Collection Tubes	5	50	200
Buffer CP*	5 ml	30 ml	120 ml
DNA Elution Buffer	1 ml	15 ml	15 ml
DNA Wash Buffer Concentrate	12 ml	40 ml	3 x 40 ml
Instruction Booklet	1	1	1

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

**Storage and Stability:** All Micro Spin™ 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 1.5 ml centrifuge tubes.
- Sterile deionized water (or TE buffer)
- Absolute (or 95%) ethanol
- Protective eye-ware

<b>IMPORTANT</b>	Wash Buffer Concentrate must be diluted with absolute ethanol as follows:	
	Sample Kit (D6292-00)	Add 18 ml ethanol
	D6292-01 D6292-02	Add 60 ml ethanol to each bottle

## Micro Spin Cycle-Pure Protocol

Please read this booklet thoroughly to ensure that you are familiar with the entire procedure. Micro Spin™ 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 microfuge tube, and add 4-5 volumes of Buffer CP.** For PCR products <200 bp add 6 volumes of Buffer CP. Vortex thoroughly to mix
3. **Apply 700 µl of sample to a Micro Spin HiBind® DNA spin-column assembled in a clean 2 ml collection tube (provided) and centrifuge in a microcentrifuge at 10,000 x g for 1 min at room temperature.** Discard the liquid. If the sample volume is more than 700 µl, load the remaining sample to the column and centrifuge as above.
4. **Wash the column by adding 600 µl of Wash Buffer diluted with absolute ethanol. Centrifuge at 10,000 x g for 1 min at room temperature.**

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

5. **Discard liquid and repeat step 5 with another 600 µl Wash Buffer.**
6. **Discard liquid and centrifuge the empty column for 1 min 10,000 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) DNA Elution Buffer directly onto the column matrix and centrifuge 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:

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

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.

### Vacuum/Spin Protocol for Micro Spin Cycle-Pure Kit

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

1. Prepare the sample by following the Protocol step 1-2.
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 750 µl DNA wash buffer, draw the wash buffer through the column by turn on the vacuum source. Repeat this step with another 750 µl DNA wash buffer.
6. Assemble the column into a 2 ml collection tube and transfer the column to a micro centrifuge. Spin 1 minute to dry the column.
7. Place the column in a clean 1.5 ml microcentrifuge tube and add 10-20µl TE or water. Stand for 1-2 minute and centrifuge 1 minute to elute DNA.

## The Micro Spin™ Gel Extraction Kit

### Kit Contents

Product Number	D6294-01	D6294-01	D6294-02
Purification	5	50	200
Micro Spin HiBind® DNA Columns	5	50	200
2 ml Collection Tubes	5	50	200
Binding Buffer	10 ml	60 ml	2 x 120 ml
DNA Elution Buffer	1 ml	15 ml	15 ml
Wash Buffer Concentrate	12 ml	40 ml	3 x 40 ml
Instruction Booklet	1	1	1

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

**Storage and Stability:** All Micro Spin™ 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.

### Micro Spin Gel Extraction 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 microfuge 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 Binding Buffer equal to 3-4 X the gel volume. Incubate the mixture at 55°C-60°C for 7 min or until the gel has completely melted. Mix by shake or vortex 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 650 µl of the DNA/agarose solution to a HiBind® DNA spin-column assembled in a clean 2 ml collection tube (provided) and centrifuge in a microcentrifuge at 8000-10,000 x g for 1 min at room temperature.** Discard the liquid. For volumes greater than 750 µl load the column and centrifuge successively, 650 µl at a time. Each HiBind® extraction column has a total capacity of 25-30 µg DNA. If the expected yield is larger, divide the sample into the appropriate number of columns.
5. **Optional: Wash the column by adding 300 µl Binding buffer. Centrifuge at 10,000 x g for 1 min.**
6. **Add 600 µl of Wash Buffer diluted with absolute ethanol into the column and wait 2-3 minutes.** Centrifuge at 10,000 x g for 1 min at room temperature to wash the column.
7. **Optional: Discard liquid and repeat step 5 with another 600 µl Wash Buffer.**
8. **Discard liquid and centrifuge the empty column for 1 min 10,000 x g to dry the column matrix. This is critical for good DNA yields.**
9. **Place column into a clean 1.5 ml microcentrifuge tube. Add 10-20 µl (depending on desired concentration of final product) DNA Elution Buffer (Supplied) or water directly onto the column matrix and centrifuge 1 min at 10,000 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.**

- 10 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:

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

Fragments greater than 500 bp in length can routinely be purified at >80% yield. Bands ranging from 50 bp to 500 bp give yields of 55%-80%. The ratio of  $(\text{absorbance}_{260})/(\text{absorbance}_{280})$  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.

## Vacuum/Spin Protocol for Gel Extraction

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

1. **Prepare the gel sample and dissolve the gel by following the Gel Extraction Protocol step 1-3.**
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. **(Optional): Wash the column with 300 µl Binding buffer by vacuum. This step is necessary if downstream application is for auto sequencing.**
6. Wash the column by adding 750 µl DNA wash buffer, draw the wash buffer through the column by turn on the vacuum source. Repeat this step with another 750 µl DNA wash buffer.
7. **Assemble the column into a 2 ml collection tube and transfer the column to a micro centrifuge. Spin 1 minute to dry the column.**
8. Place the column in a clean 1.5 ml microcentrifuge tube and add 10-20µl Elution Buffer or water. Stand for 1-2 minute and centrifuge 1 minute to elute DNA.

## Micro Spin DNA Clean-Up Kit

### Kit Components

Product No.	D6296-00	D6296-02	D6296-02
Purification	5	50	200
Micro Spin HiBind DNA Column	5	50	200
2ml Collection Tubes	5	50	200
Buffer DP	5 ml	30ml	120ml
DNA Wash Buffer	12 ml	40 ml	3 x 40 ml
Elution Buffer	1 ml	15 ml	15 ml
Instruction Booklet	1	1	1

**Storage and Stability:** All Micro Spin™ 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 Binding Buffer is capped tightly when not in use.

1. Add 400 µl volume of DP buffer to the enzymatic reaction and mix thoroughly with pipetting or vortex. The maximum volume of the reaction can be processed per column is 120 µl.
2. Place a Micro Spin 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 12,000 x g for 1 minute to bind DNA.
5. Discard the flow-through and reuse the collection tubes for next step.
6. Add 600 µl of DNA wash buffer to the Micro Spin HiBind™ DNA column and spin at 12,000 x g for 1 minute. Discard the flow-through and place the column back into same 2ml collection tube.
7. Centrifuge at maximum speed for 1 minute to dry the column. Discard the 2 ml collection tubes.
8. Place the Micro Spin HiBind™ DNA column into a clean 1.5 ml micro centrifuge tube.
9. Add 10-20 µl Elution Buffer or water to the center of the membrane, incubate for 1 minute.
10. Centrifuge at 12,000 x g for 1 minute to elute DNA.

### Vacuum/Spin Protocol for Gel Extraction

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

1. Add 400 µl volume of DP buffer to the enzymatic reaction and mix thoroughly with pipetting or vortex. The maximum volume of the reaction can be processed per column is 120 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 DNA wash buffer, draw the 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 1 minute to dry the column.**
7. Place the column in a clean 1.5 ml microcentrifuge tube and add 10-20µl Elution Buffer or water. Stand for 1-2 minute and centrifuge 1 minute to elute DNA.

### Troubleshooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Too little Buffer CP, Binding buffer or DP added to sample.	Add more Buffer CP, DP 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.	Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare 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 in steps 4 and 5. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantitation.
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.

## Ordering Information

Product No.	Product Name	Description
D6493-01/02 D6492-01/02	Cycle-Pure Kit	PCR product purification, Q-Column format & V-column format.
D1043-01/02	E-Z 96 Cycle-Pure Kit	96 well format PCR purification
D6537-01/02 D6538-01/02	DNA Probe Purification Kit	DNA probe purification, Q-column & V-column format
D2561-01/02	Poly-Gel DNA Purification Kit	Isolate DNA from polyacrylamide gel
D2501-01/02 D2500-01/02	Gel Extraction Kit	Agarose gel extraction using spin column technology
D2510-01/02	Ultra-Sep Gel Purification Kit	Agarose gel extraction using silica beads.
R6376-01/02	Poly-Gel RNA Purification Kit	Isolate RNA from polyacrylamide gel
R6537-01/02 R6538-01/02	RNA Probe Purification Kit	RNA probe purification, Q-column & V-column format

\* All OBI products available with size if 50 preps and 200 preps. Product number end with "-01" represent 50 preps kit and "-02" represent 200 preps kit.