MicroElute® Cycle-Pure & Gel Extraction Kit

Revision No: 1.0

Quick Guide

Please visit www.omegabiotek.com for a downloadable user manual containing additional protocols, troubleshooting tips, and ordering information.

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
CP Buffer	5 mL	30 mL	120 mL
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

Supplied by user:

- Microcentrifuge capable of at least 13,000*g*
- Incubator capable of 60°C
- Vortexer
- 100% ethanol
- 3M NaOH
- Sterile deionized water
- Optional: 5M sodium acetate, pH 5.2

Before starting:

- Prepare SPW Buffer according to the instructions on the bottles.

DNA Purification from Agarose Gels - Centrifugation Protocol

LYSE

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. Remove 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.

BIND

4. Add 1 volume XP2 Binding Buffer. Incubate at 60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes. Monitor the pH of the Gel/Binding Buffer mixture after the gel has dissolved. If the color of the mixture becomes orange or red, add 5 µL 5M sodium acetate (pH 5.2). The color of the Gel/Binding Buffer mixture should be light yellow.
5. Insert a MicroElute® LE DNA Column into a 2 mL collection tube 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.

6. Transfer no more than 700 µL DNA/agarose solution from Step 4 to the MicroElute® LE DNA Column.
7. Centrifuge at 10,000*g* for 1 minute at room temperature. Discard the filtrate and reuse collection tube.
8. Repeat Steps 6-7 until all of the sample has been transferred to the column.
9. Add 300 µL XP2 Binding Buffer. Centrifuge at maximum speed (≥13,000*g*) for 30 seconds at room temperature. Discard the filtrate and reuse collection tube.

WASH

10. Add 700 µL SPW Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at maximum speed for 1 minute at room temperature. Discard the filtrate and reuse collection tube.

Optional: Repeat Step 10 for a second SPW Buffer step for any salt sensitive downstream applications.

WASH

11. Centrifuge the empty MicroElute® LE DNA Column for 2 minutes at maximum speed to dry the column matrix. It is important to dry the column matrix before elution. Residual ethanol may interfere with downstream applications.
12. Transfer the MicroElute® LE DNA Column to a clean 1.5 mL microcentrifuge tube.

ELUTE

13. Add 10-20 µL Elution Buffer or sterile deionized water (pH 8.5) directly to the center of the column membrane. Let sit at room temperature for 2 minutes. Centrifuge at maximum speed for 1 minute. An optional second elution will yield any residual DNA, though at a lower concentration.
14. Store DNA at -20°C.

DNA Purification from Amplified Reactions - Centrifugation Protocol

LYSE

1. Perform agarose gel/ethidium bromide electrophoresis to to analyze PCR product. Determine the volume of your PCR reaction.
2. Transfer the sample into a clean 1.5 mL microcentrifuge tube (not provided).

BIND

3. Add 5 volumes CP Buffer. For fragments <200 bp, add 5 volumes CP Buffer and 0.4 volumes 100% isopropanol. Volume refers to the size of your PCR reaction.

   Note: 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.
4. Vortex to mix thoroughly. Briefly centrifuge to collect any drops from the inside of the lid.
5. Insert a MicroElute® LE DNA Column into a 2 mL collection tube 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.

6. Transfer the sample from Step 4 to the MicroElute® LE DNA Column.
7. Centrifuge at maximum speed (≥13,000g) for 1 minute at room temperature. Discard the filtrate and reuse the collection tube.

WASH

8. Add 700 µL SPW Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse the collection tube.
9. Repeat Step 8 for a second SPW Buffer step.
10. Centrifuge the empty MicroElute® LE DNA Column at maximum speed for 2 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications.
11. Transfer the MicroElute® LE DNA Column to a clean 1.5 mL microcentrifuge tube.

ELUTE

12. Add 10-20 µL Elution Buffer, TE Buffer, or sterile deionized water (pH 8.5) directly to the center of the column membrane. Let sit at room temperature for 2 minutes. Centrifuge at maximum speed for 1 minute. An optional second elution will yield any residual DNA, though at a lower concentration.
13. Store DNA at -20°C.

If use of a vacuum is desired, please refer to the MicroElute® Cycle Pure - Vacuum Protocol or MicroElute® Gel Extraction - Vacuum Protocol starting on Page 13 or Page 19 of the full manual found at https://omegabiotek.com.