MicroElute® Cycle-Pure Kit

Revision No: 4.1

Quick Guide

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

Product	D6293-00	D6293-01	D6293-02
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
DNA Wash 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*
- Vacuum manifold
- Vortexer
- 1.5 mL microcentrifuge tubes
- 100% ethanol
- 3M NaOH
- Sterile deionized water
- For fragments < 200 bp: 100% isopropanol

Before starting:

- Prepare DNA Wash Buffer according to the instructions on the bottles.

DNA Purification from Amplified Reactions - Centrifugation Protocol

LYSE

1. Perform agarose gel/ethidium bromide electrophoresis 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).
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.

BIND

5. Insert a MicroElute® LE DNA Column into a 2 mL Collection Tube (provided) 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 (≥13,000*g*) for 1 minute at room temperature. Discard the filtrate and reuse the collection tube.

WASH

8. Add 700 µL DNA Wash 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 DNA Wash Buffer wash 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.

ELUTE

11. Transfer the MicroElute® LE DNA Column into a clean 1.5 mL microcentrifuge tube (not provided).
12. Add 10-20 µL Elution Buffer, TE Buffer, or sterile deionized water directly to the center of column matrix. Let sit at room temperature for 2 minutes. Centrifuge at maximum speed for 1 minute. This represents approximately 80-90% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.
13. Store DNA at -20°C.

DNA Purification from Amplified Reactions - Vacuum Protocol

LYSE

1. Perform agarose gel/ethidium bromide electrophoresis 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).
3. Add 5 volumes CP Buffer. For fragments < 200 bp add 5 volumes CP Buffer and 0.4 volumes 100% isopropanol.

   Note: Volume refers to the size of your PCR reaction. 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.

BIND

5. Prepare the vacuum manifold according to manufacturer's instructions. Connect the MicroElute® LE DNA Column to the manifold 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. Switch on vacuum source to draw the buffer through the column.
3. Turn off the vacuum.
4. Add 500 µL sterile deionized water to the MicroElute® LE DNA Column.
5. Switch on vacuum source to draw the water through the column.
6. Turn off the vacuum.

6. Transfer the entire sample from Step 4 to the MicroElute® LE DNA Column. Switch on vacuum source to draw the sample through the column. Turn off the vacuum.

WASH

7. Add 700 µL DNA Wash Buffer diluted with 100% ethanol (see the bottle for instructions). Switch on vacuum source to draw the DNA Wash Buffer through the column. Turn off the vacuum.
8. Repeat Step 7 for a second DNA Wash Buffer wash step.
9. Transfer the MicroElute® LE DNA Column into a 2 mL Collection Tube.
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.

ELUTE

11. Transfer the MicroElute® LE DNA Column into a clean 1.5 mL microcentrifuge tube (not provided).
12. Add 10-20 µL Elution Buffer, TE Buffer, or sterile deionized water directly to the center of column matrix. Let sit at room temperature for 2 minutes. Centrifuge at maximum speed for 1 minute. This represents approximately 80-90% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.
13. Store DNA at -20°C.