Biomiga Inc.

The Inventor of EZgene™ and ViraTrap™ Systems

EZgene® 96 PCR Purification Kit – User Manual

Introduction

The EZgene® family of products provides innovative solutions that simplify the extraction and purification of nucleic acids from a wide range of biological samples. Central to this system is a proprietary matrix that selectively and reversibly binds DNA or RNA under optimized conditions, allowing proteins and other contaminants to be efficiently removed. Purified nucleic acids are subsequently eluted using deionized water or a low-salt buffer.The **EZgene® 96 PCR Purification Kit** is a convenient and reliable system designed for high-throughput purification of up to 96 PCR products simultaneously. Using EZgene technology, the kit efficiently recovers DNA fragments ranging from **100 bp to 10 kb**, removing primers, nucleotides, and polymerases with typical yields exceeding **80%**. Following rapid wash steps, DNA is eluted and ready for downstream applications such as **T-A cloning, PCR sequencing, restriction digestion, and labeling reactions**. The protocol eliminates the need for organic extractions or alcohol precipitations, ensuring safe and rapid parallel processing.

Each well of the EZgene® 96 DNA Plate can bind up to **~20 µg of DNA**.

Kit Contents

Product Number	**DC3812-00**	**DC3812-01**	**DC3812-02**
Purifications	1 × 96	4 × 96	24 × 96
96-Well DNA Plates	1	4	24
Elution Plates	1	4	24
2 mL Deep-Well Plates*	1	2	4
Buffer GC	60 mL	120 mL	480 mL
DNA Wash Buffer (Concentrate)	50 mL	50 mL	2 × 200 mL
Elution Buffer	15 mL	60 mL	300 mL
Sealing Films	2	8	48

Storage and Stability

All EZgene® 96 Cycle-Pure Kit components are stable for **at least 12 months** from the date of purchase when stored at **22–25 °C**.
Crystals may form in Buffer GC under cooler conditions; warm to **37 °C** to fully dissolve before use.

Preparing Reagents

DNA Wash Buffer Preparation

Dilute the DNA Wash Buffer Concentrate with absolute ethanol (96–100%) as follows:

Kit	Ethanol to Add
DC3812-01	Add 200 mL ethanol
DC3812-02	Add 800 mL ethanol
DC3812-03	Add 800 mL ethanol to each bottle

Store diluted DNA Wash Buffer at **room temperature**.

Equipment Required for Vacuum Protocol

- Vacuum manifold (Biomiga VAC-03 recommended)
  Compatible alternatives: Qiagen QIAvac 96, Macherey-Nagel NucleoVac 96
- Vacuum flask
- Vacuum tubing
- Vacuum source (refer to pressure conversion tables)

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96-Well PCR Purification Vacuum Protocol

All steps should be performed at **room temperature**.

Materials Provided by User

- Vacuum manifold compatible with 96-well plates
- Absolute ethanol (96–100%)
- Plate seals and lids
- Protective eyewear

Procedure

1. Analyze PCR products by agarose gel electrophoresis.
2. Add **1 volume Buffer GC** to **1 volume PCR product** and mix thoroughly.
    - For fragments <200 bp, add **2 volumes Buffer GC**.
3. Assemble the vacuum manifold according to the manufacturer's instructions. Place a deep-well plate inside the manifold and position the 96-well DNA Plate on top. Seal unused wells.
4. Apply vacuum to draw samples through the membrane. Discard flow-through.
5. Add **800 µL DNA Wash Buffer** to each well.
6. Apply vacuum and discard flow-through.
7. Repeat wash with another **800 µL DNA Wash Buffer**.
8. Apply vacuum for **10–15 minutes** to fully dry the membrane.
9. Remove the DNA plate and gently tap on absorbent paper to remove residual ethanol.
10. Place an elution plate beneath the DNA plate.
11. Add **60–100 µL Elution Buffer (10 mM Tris, pH 8.5)** directly to the membrane and apply vacuum for **5 minutes** to elute DNA.

Optional: Reapply eluate for a second elution to increase yield.

96-Well PCR Purification Spin Protocol

All steps are performed at **room temperature**.

1. Analyze PCR products by agarose gel electrophoresis.
2. Add Buffer GC as described above and mix thoroughly.
3. Place the 96-well DNA Plate onto a deep-well plate and load samples.
4. Centrifuge at **3,000–4,000 × g for 10 minutes**.
5. Discard flow-through.
6. Wash twice with **800 µL DNA Wash Buffer**, centrifuging after each wash.
7. Dry the membrane by centrifugation at **4,000 × g for 15–20 minutes**.
8. Transfer the DNA plate to an elution plate, add **60–100 µL Elution Buffer**, and centrifuge at **4,000 × g for 10 minutes**.

Troubleshooting Guide

Problem	Possible Cause	Solution
Low DNA yield	Insufficient Buffer GC	Increase Buffer GC volume as recommended
Low elution efficiency	Elution buffer pH <7.5	Use fresh Elution Buffer
High A260 readings	Trace contaminants	Ensure thorough washing; confirm yield by gel
DNA floats from well	Residual ethanol	Extend drying step before elution

For additional assistance, contact call us at **858-603-3219**