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Introduction

The E-Z 96 X-press system provide and fast and simple plasmid purification method for routine molecular biology applications. This innovative technology significantly reduce the process time comparing the traditional alkaline lysis-based method. Plasmid isolation can be completed in less than 10 minutes.

The E-Z 96 X-press Plasmid DNA Kit is based upon proprietary technology to provide a fast and consistent method for molecular grade plasmid isolation. This kit uses a single solution to resuspend, lyse and binding. After the cell lysis, plasmid is bound onto the membrane of a E-Z 96 X-press Plate. After a simple wash step, the bound plasmid DNA is eluted with from the plate with low salt buffer and ready to use for downstream applications. Yield varies depend on the bacterial strain, growing condition, media and copy number of plasmid. Typically, a 1.5 ml overnight bacterial culture with LB medium with high-copy number plasmid will yield 3-8 µg plasmid DNA.

Storage and Stability

All E-Z 96 X-press Plasmid Kit components are guaranteed for at least 12 months from the date of purchase when stored by following the instruction. All components except enzyme (RNase A and lysozyme) should be store at room temperature (22-25°C). Store RNase A and lysozyme at 2-8°C.

Binding Capacity

Each well of E-Z 96 X-press plate can bind approximately 25 µg Plasmid DNA.

Kit Contents

Product Number	D1047-00	D1047-01	D 1047-02
Purification	1 x 96	4 x 96	20 x 96
E-Z 96 X-press Plate	1	4	20
96-Well Deep-well Plate (2 mL)	1	4	20
Collection plate (300µl)	1	4	20
XCL Buffer	3 mL	20 mL	60 mL
DLW Buffer	1.3 mL	13 mL	39 mL
Lysozyme	3 mg	30 mg	120 mg
RNase A (10mg/ml)	100 µL	400 µL	1.6 mL
Elution Buffer	1.0 mL	10 mL	25 mL
Instruction Booklet	1	1	1

Before Starting

Briefly examine this booklet and become familiar with each step. Prepare all components and have the necessary materials ready before starting.

- **Prepare the XCL Lysis Buffer:** 1.Briefly spin the RNase A vial to collect any liquid drops. Transfer the entire volume of RNase A into the vial containing lysozyme and resuspend the lysozyme completely by pipetting up and down. Transfer RNase A/lysozyme mix into the bottle which contains XCL Buffer. **Store completed XCL Lysis Buffer Mixture at 2-8C.**

- **Prepare DLW Buffer by adding isopropanol as following**

D6947-00	**Add 2.7 ml Isopropanol (100%)**
D6947-01	**Add 27 ml Isopropanol (100%)**
D6947-02	**Add 81 ml Isopropanol (100%)**

E-Z 96 X-press Plasmid Vacuum Protocol

Materials to Be Provided by User

Have the following reagents and supplies ready:

- Tabletop with swinging-bucket rotor capable of 3,000 x g
- Vacuum Manifold (Cat# Vac-03)
- Nuclease Free 1.5 and 2.0 mL Centrifuge Tubes
- Ice
- Isopropanol (100%)
- Vortex

1. Pick up a single colony from a fresh streaked selective plate and Inoculate 2-3 mL LB medium containing the appropriate selective antibiotic. Incubate 14-16 hours at 37°C with vigorous shaking until an OD_{600} of 2.0-4.0 is achieved.

2. **Pellet 1-2 mL bacterial culture in a 2.0 ml 96-well deep-well plate by centrifugation at 3000 x g for 15 min at room temperature**. **Note:** Do not use biomass large than 3.0. For example: If the OD600 of the culture is 2.0, use a 1.5 ml bacterial culture.

3. **Discard medium and remove any remaining liquid in the tube by using a pipettor or inverting the tube on an absorbent paper for 1 minute .**

4. **Add 500µl ICE-COLD XCL Lysis Buffer contains RNase A and lysozyme to each well of the plate.**

   Note: XCL Lysis Buffer must be **ice cold** to achieve maximum plasmid yield**.**

5. **Completely resuspend the cell pellet by vortexing the plate at maximum speed for 30-60 seconds.**

   Note: It is critical to fully resuspend the cell pellet to obtain optimized DNA yield.

6. **Incubate at room temperature for 3-5 minutes.** The cell lysate should be nonviscous and slightly cloudy after incubation.

7. **Assemble the vacuum manifold according to the manufacturer's**

instruction. For using Omega Bio-Tek's Vac-03: 1). Place E-Z 96 X-press Plate on the top plate of manifold; 2). Place a waste collection tray or 2 ml deep well plate inside the manifold base; 3). Place the top plate of manifold over the base, the deep well plate or waste collection tray now should be positioned under the E-Z 96 X-press Plate. Seal the unused wells of E-Z 96 Xpress Plate with sealing film.

8. **Transfer the entire cell lysate into E-Z 96 X-press Plate. Apply vacuum until all the lysate pass through the plate.**

9. **Apply the vacuum until all the lysate passes through the E-Z 96 X-press Plate .** Turn off the vacuum.

10. **Add 500µl DLW Buffer (diluted with isopropanol) into Each well of E-Z 96 X-press Plate.**

11. **Apply the vacuum until all the lysate passes through the Plate .** After all the liquid pass through the plate, continue the vacuum for additional 3 minutes. Turn off the vacuum.

12. **Remove the E-Z 96® DNA Plate from the vacuum manifold, then vigorously tap the plate on a stack of absorbent paper towels until no drops come out.** Remove any residual moisture from the tip ends of the DNA plate with clean absorbent paper towels.

13. **Place the E-Z 96 X-press Plate back to the vacuum manifold and apply the maximum vacuum for another 10 minutes.** This step will evaporate any remaining ethanol from membrane.

14. Turn off the vacuum. Assemble the vacuum manifold by place a new 300 µl 96-well collection plate (provided) inside the base of manifold. If Omega manifold (Vac-03) is used in this procedure, a used E-Z 96 X-press Plate or a 800 µl plate should be placed under the 300 µl collection plate as a support to give the collection plate a proper position.

15. **Add 100-150 µl Elution Buffer (10mM Tris-HCl, pH 8.5) or sterile water** to each well of the E-Z 96® X-press Plate, let stand for 2 minutes. Apply maximum vacuum for 5-10 minutes to elute DNA from the plate. Turn off the vacuum and ventilate the manifold slowly.

E-Z 96 X-press Plasmid Spin Protocol

Materials to Be Provided by User

Have the following reagents and supplies ready:

- Tabletop with swinging-bucket rotor capable of 3,000 x g
- Vacuum Manifold (Cat# Vac-03)
- Nuclease Free 1.5 and 2.0 mL Centrifuge Tubes
- Ice
- Isopropanol (100%)
- Vortex

1. **Process the cell lysate by following step 1-6 from vacuum protocol on page 4-5.**

2. **Place a E-Z 96 X-press plate on top of a 96 deep-well plate. Transfer the cell lysate to each well of E-Z 96 X-press plate.**

3. **Centrifuge at 3000 x g for 5 minutes at room temperature. Discard the flow-through and re-use the deep well plate.**

4. **Add 500µl DLW Buffer (diluted with isopropanol) into Each well of E-Z 96 X-press Plate.**

5. **Centrifuge at 3000 x g for 15 minutes at room temperature. Discard the flow-through and re-use the deep well plate.**

6. **Place the E-Z 96 X-press Plate on a new** a new 300 µl 96-well collection plate (provided).

7. **Add 100-150 µl Elution Buffer (10mM Tris-HCl, pH 8.5) or sterile water** to each well of the E-Z 96® X-press Plate, let stand for 2 minutes.

8. Centrifuge at 3000 x g for 5 minutes to elute plasmid DNA.

Trouble Shooting Guide

Problem	Likely Cause	Suggestions
Low DNA yields	Poor cell lysis	Do not use more than 3.0 OD of cell mass culture. Excess biomass can cause incompletely cell lysis and leads to low DNA yield. Ensure the XCL Buffer incubation time is more than 3 minutes. Increase the incubation time if necessary. XCL Buffer need to be ice-cold for obtains maximum yield.
	Storage of XCL Buffer is not correct or old.	Make sure the XCL buffer is stored at 2-8 °C
	Low copy-number plasmid used	Such plasmids may yield as little as 0.2µg DNA from a 1 mL overnight culture.
	DLW Buffer is prepared incorrectly.	Ensure correct amount of isopropanol is added to the DLW Buffer.
	Binding condition is altered due to vector or bacterial stain	Add 150µl isopropanol to the cell lysate before loading to the X-press Plate.
No DNA eluted.	System is overloaded	ensure the culture has OD600 of 2.0-4.0.
	DLW Buffer is prepared incorrectly.	Ensure correct amount of isopropanol is added to the DLW Buffer.
Optical densities do not agree with DNA yield on agarose gel.	Trace contaminants eluted from X-press Plate increase A_{260}.	Make sure to wash X-press Plate as instructed. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantization.

RNA visible on agarose gel.	RNase A not added to XCL Buffer	Add 1 vial of RNase to the bottle of XCL Buffer
Plasmid DNA floats out of well while loading agarose gel	Ethanol not completely removed from X-press Plate following wash steps.	Dry the X-press Plate before the elution