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Introduction

The E.Z.N.A.™ 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.N.A.™ X-press Plasmid DNA Mini 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 X-press spin column. After a simple wash step, the bound plasmid DNA is eluted with from the column 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.N.A.™ X-press Plasmid DNA Mini 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 X-press spin column can bind approximately 25 µg Plasmid DNA.

Kit Contents

Product Number	D6947-00	D6947-01	D 6947-02
Purification	5 Preps	50 Preps	200 Preps
X-press Spin columns	5	50	200
2 mL Collection Tubes	5	50	200
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. N.A.™ X-press Plasmid Protocol

Materials to Be Provided by User

Have the following reagents and supplies ready:

- Tabletop micro-centrifuge capable of 13,000 x g
- 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 centrifuge tube by centrifugation at maximum speed (≥13,000 × g) for 1 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 which contains RNase A and lysozyme.**

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

5. **Completely resuspend the cell pellet by vortexing 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. **Transfer the entire cell lysate into a X-press spin column inserted in a 2 ml collection tube.**

8. **Centrifuge at maximum speed (≥13,000 x g) for 1 minute.**

9. **Discard the flow-through liquid and re-use the collection tube.**

10. **Add 500µl DLW Buffer (diluted with isopropanol) into the X-press spin column.**

11. **Centrifuge at maximum speed (≥13,000 x g) for 1 minute.**

12. **Discard the flow-through liquid and re-use the collection tube.**

13. **Place the empty X-press spin column back into the 2 ml collection tube. Centrifuge at maximum speed (≥13,000 x g) for 1 minute to dry the column.**

14. **Transfer the X-press spin column into a new 1.5 ml centrifuge tube. Add 50 µL Elution Buffer (10mM Tris Hcl pH 8.5) directly onto the center of the membrane.**

15. **Centrifuge at maximum speed (≥13,000 x g) for 1 minute to elute the 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 column.
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 column increase A_{260}.	Make sure to wash column 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 column following wash steps.	Dry the column before the elution