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Introduction

The E-Z 96 Swift Plasmid Kit is designed for isolate of high copy number plasmid in 96 well format. The uses a novel lysis chemistry that eliminates the need for a lysate clearing step. Pelleted bacterial cells are resuspended and then lysed. The lysate is neutralized using an optimized buffer, which leads to only a small amount of precipitated cellular components. Centrifugation or filtration of the lysate is not required. Isopropanol is added in order to optimize DNA binding conditions on the Swift 96 Plate. Plasmid DNA binds to the activated membrane, which is subsequently washed with an ethanol-containing buffer and then dried. Plasmid DNA is eluted using the elution buffer provided in the kit. The DNA obtained is highly suited for automated fluorescent sequencing, and other routine applications, such as restriction digestion and PCR. Please note that the Swift 96 procedure is suitable for use with high-copy plasmids only.

Storage and Stability

All E-Z 96® Swift Plasmid Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: The mixture of Solution I/RNase A at 4°C; all other material at 22-25°C. Store Solution ll tightly capped.

Kit Contents

Product No.	D2153-00	D2153-01	D2153-02
Purification times	2x 96 Preps	4 x 96 Preps	20 x 96 Preps
E-Z 96® Swift Plate	2	4	20
96-Well Collection Plate (2.0 mL)	2	2	4
Elution Plate (300 µL)	2	4	20
SealPlate Sealing film	8	16	80
Solution I	50 mL	100 mL	2 x 200 mL
Solution II	50 mL	100 mL	2 x 200 mL
Buffer SW3	50 mL	100 mL	2 x 200 mL
Elution Buffer	50 mL	100 mL	2 x 200 mL
RNase A	300 µL	600 µL	2 x1.2 mL
Instruction Booklet	1	1	1

* The 2mL 96-well collection plates are reusable. See page 8 for detailed instructions.

Before Starting

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

1. Add vial of RNase A to bottle of Solution I and Store at 4ºC.

2. Prepare one bottle of 70% ethnaol.

E-Z 96® Swift Plasmid Vacuum Manifold Protocol

Materials Supplied By User

- Vacuum pump or vacuum aspirator capable of achieving a vacuum of 300-400mbar
- Standard vacuum manifold (i.e: Omega Product #VAC-03)
- Optional: Racked Microtubes (for elution)

1. Culture Volume: **Innoculate 1.25 mL LB or 2 x YT medium placed in a 96-well 2 mL culture block with *E.coli* carrying desired plasmid and grow at 37°C with agitation (180-300 rpm) for 16-24 h.** It is strongly recommended that an endA negative strain of *E.coli* be used for routine plasmid isolation. Examples of such strains include DH5α® and JM109®.

2. Seal the plate with sealing film and pellet bacteria by **centrifugation at 1500 x g for 10 minutes** in a swinging-bucket rotor at room temperature.

3. Remove the sealing film and discard supernatant into a waste container. Dry the plate by tapping the inverted block firmly a paper towel to remove excess media. **Add 150 µl Solution I/RNase A to the bacterial pellet in each well of the plate.** Resuspend cells completely by vortexing and/or pipetting. No cell clumps should be visible after resuspension of the pellet. Complete resuspension of cell pellet is vital for obtaining good plasmid yields.

4. **Add 150 µl Solution II into each well and mix throughly by gently shaking and rotating the plate for 1 minute to obtain a cleared lysate.** A 3-4 min incubation at room temperature may be necessary. The solution should become viscous and slightly clear. Avoid vigorous mixing as doing so will shear chromosomal DNA and lower plasmid purity. (Store Solution II tightly capped when not in use.)

5. **Add 150 µl Buffer SW3 to each well.** Dry the top of the plate with a paper tower. Seal the plate with sealing film and mix by shaking or vortexing the plate until a flocculent white precipitate forms.

6. **Remove the sealing film and add 300 ul isopropanol to each well.** Dry the top of the plate with a paper tower. Seal the plate with Sealing film and mix by shaking the plate for 1 min. Inversions of the plate are not required and may cause leakage of the isopropanol.

7. **Assemble the vacuum manifold**: Assemble the vacuum manifold: 1). Place E-Z 96® Swift Plate in the top plate of manifold; 2). Place the waste tray inside the manifold base.

8. **Transfer the crude lysate from step 6 into the wells of E-Z 96® Swift Plate**. Allow the lysate to stand for 2 minutes.

9. **Apply the vacuum until all the lysate passes through**. Adjust the vacuum as necessary to control the flow rate as 1-2 drop per second.

   Note: Apply vacuum too quicky can cause the clogging of the plate.

10. **Add 1 ml of 70% ethanol to each well**, then apply the vacuum until all the liquid passes through.

11. **Place a new E-Z 96® Swift Plate on top of a 96-well 2 ml plate.** Place the Swift/Collection plates complex into centrifuge's swing-bucket rotor with adapter for deep well plate. Centrifuge at maxi speed (<5,000 x g) for 15 minutes **at room temperature to dry the plate.**

12. Place the E-Z 96® DNA Plate on top of a new 96-well Elute Plate.

13. **Add 50-100 µl Elution Buffer (10mM Tris-HCl, pH 8.5) or sterile water to each well of the E-Z 96® DNA Plate**. Incubate at room temperature for 3 minutes.

14. **Centrifuge at 2,000 x g for 5 minutes to elute DNA.** This represents approximately 75-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

15. Store eluted DNA sample at -20°C.

E-Z 96® Swift Plasmid Spin Protocol

Materials Supplied By User

- Centrifuge with swinging-bucket rotor at room temperature capable of 4,000 x g
- Adapter for 96-well collection plate
- DNase/RNase-free 96-well 2.0 mL plates
- Absolute (96%-100%) ethanol

1. **Culture Volume:** Innoculate 1.25 mL LB/antibiotic(s) medium placed in a 96-well 2mL culture plate/block with *E.coli* carrying desired plasmid and grow at 37°C with agitation (180-300 rpm) for 20-24 h**.** It is strongly recommended that an endA negative strain of E.coli be used for routine plasmid isolation**.** Examples of such strains include DH5α® and JM109®.

2. **Seal the plate with sealing film and pellet bacteria by centrifugation at 1,500 x g for 10 minutes in a swinging-bucket rotor at room temperature**.

3. **Discard supernatant into a waste container**. **Dry the plate by tapping the inverted block firmly a paper towel to remove excess media. Add 150 µl Solution I/RNase A to the bacterial pellet in each well of the plate**. Resuspend cells completely by vortexing and/or pipetting. No cell clumps should be visible after resuspension of the pellet. Complete resuspension of cell pellet is vital for obtaining good plasmid yields.

4. **Add 150 µl Solution II into each well** and mix by gently shaking and rotating the plate for 1 minute to obtain a cleared lysate. A 2-5 min incubation at room temperature may be necessary. The solution should become viscous and slightly clear.
   Note: Avoid vigorous mixing as doing so will shear chromosomal DNA and lower plasmid purity. (Store Solution II tightly capped when not in use.)

5. **Add 150 µl Solution III to each well.** Dry the top of the plate with a paper tower. Seal the plate with sealing film and mix by shaking or vortexing the plate until a flocculent white precipitate forms.

6. **Remove the sealing film and add 300 ul of isopropanol to each well.** Dry the top of the plate with a paper tower. Seal the plate with Sealing film and mix by shaking the plate for 1 min. Inversions of the plate are not required and may cause leakage of the isopropanol.

7. **Place a new E-Z 96® Swift Plate on top of 2 ml Collection Plate.**

8. Transfer the crude lysate from step 6 into E-Z 96® Swift Plate.

9. **Place the Swift/Collection plates complex into centrifuge's swing-bucket rotor with adapter for deep well plate.** Centrifuge at 2,000-5,000 x g for 3 minutes.

10. **Discard the flow-through liquid and re-use the deep well collection plate for next step**.

11. **Add 1 ml 70% ethanol Buffer to each well of E-Z 96® Swift Plate**. **Centrifuge at 2,000-5,000 x g for 3 minutes. Discard the flow-through and reuse the collection plate for next step.**

12. **Place the swift plate back on top of collection plate. Centrifuge at maxi speed for 15 min at room temperature for 15 min to dry the plate.**

13. **Place the E-Z 96® DNA Plate on top of a new 96-well Elute Plate.**

14. **Add 50-100 µl Elution Buffer (10mM Tris-HCl, pH 8.5) or sterile water to each well of the E-Z 96® DNA Plate**. Incubate at room temperature for 3 minutes.

15. **Centrifuge at 2,000-5,000 x g for 5 minutes to elute DNA.** This represents approximately 75-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

16. Store eluted DNA sample at -20°C.

Cleaning of 2mL 96-Well Collection Plates

The 2mL 96-well collection plates are reusable. To avoid cross-contamination, rinse the plates throughly with tap water after each use. Rinse with 0.5 M HCl for 5minutes, then rinse throughly with distilled water. 2mL 96-well collection plates can also be autoclaved after washing.

Troubleshooting Guide

Low DNA yields

a) Poor Cell Lysis	Cells may not have been dispersed adequately prior to the addition of Solution II. Make sure to vortex cell suspension to completely disperse.
	Solution II if not tightly closed, may need to be replaced. Prepare as follows: 0.2 N NaOH, 1% SDS.
b) Bacterial Clone is overgrown or not fresh.	Do not incubate cultures for more than 24 hr at 37°C. Storage of cultures for extended periods prior to plasmid isolation is detrimental.
c) Low elution efficiency	The pH of Elution Buffer or water must be ≥8.0

High molecular weight DNA contamination of product.

e) Over mixing of cell lysate upon addition of Solution II.	Do not vortex or mix aggressively after adding Solution II.
f) Culture overgrown	Overgrown culture contains lysed cells and degraded DNA. Do not grow cell for longer than 24hours.

RNA visible on agarose gel

h) RNase A not added to Solution I.	Check that RNase A provided with the kit has been used. If Solution I is more than 6 months old, add more RNase A.

Plasmid DNA floats out of well while loading agarose gel

j) Ethanol has not completely been removed from E-Z 96 DNA Plate following wash steps.	Ensure applying the maximum vacuum. Place the DNA Plate into a vacuum oven to further dry the plate.

Plasmid DNA will not perform in downstream application

Traces of ethanol remain on plate prior to elution.	Bake the plate as instructed in step 17 to further dry. Ethanol precipitation may be required following elution.
DNA is permanently denatured	Cell lysis process should not be over 5 minutes. It may cause DNA permanently denatured.