Zyppy™ Plasmid Miniprep Kit

Zymo Research

Abstract

The **Zyppy™ Plasmid Miniprep Kit** features a **Pellet-Free™** modified alkaline lysis method that bypasses bacterial culture centrifugation and resuspension steps common to classical plasmid preparation procedures. Simply add the uniquely formulated **7X Lysis Buffer** directly to your bacterial culture, neutralize, then purify using our Fast-Spin column technology (alternatively, the samples may be processed by the classical centrifugation method). Additionally, the innovative colored buffers included in the kit permit error-free visualization identification of complete bacterial cell lysis and neutralization.

Citation: Zymo Research Zyppy™ Plasmid Miniprep Kit. **protocols.io**
dx.doi.org/10.17504/protocols.io.gi3bugn
Published: 03 Dec 2016

Guidelines

Product Contents

Zyppy™ Plasmid Miniprep Kit	D4036 50 preps.	D4019 100 preps.	D4020 400 preps.	D4037 800 preps.	Storage Temperature
7X Lysis Buffer*[1] (Blue)	6 ml	12 ml	48 ml	2 x 48 ml	Room Temp.
Neutralization Buffer*[2] (Yellow)	20 ml	40 ml	160 ml	2 x 160 ml	4-8 ºC
Endo-Wash Buffer	15 ml	30 ml	120 ml	2 x 120 ml	Room Temp.
Zyppy™ Wash Buffer (concentrate)[3]	6 ml	12 ml	48 ml	2 x 48 ml	Room Temp.
Zyppy™ Elution Buffer	5 ml	5 ml	20 ml	2 x 20 ml	Room Temp.
Zymo-Spin™ IIN Columns	50	2 x 50	8 x 50	16 x 50	Room Temp.
Collection Tubes	50	2 x 50	2x 200	4 x 200	Room Temp.
Instruction Manual	1	1	1	1	-

Note – Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

[1] The 7X Lysis Buffer may have precipitated during shipping. To completely resuspend the buffer, incubate the bottle at 30 – 37 °C for 30 minutes and mix by inversion. DO NOT MICROWAVE.

[2] Neutralization Buffer contains RNase A at a concentration of 200 µg/ml.

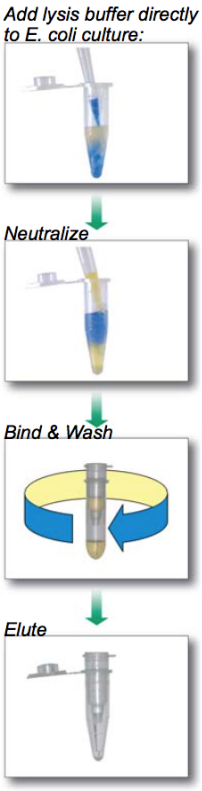
[3] Add 24 ml of 100% ethanol (26 ml of 95% ethanol) to the 6 ml Zyppy™ Wash Buffer concentrate (D4036), 48 ml of 100% ethanol (52 ml of 95% ethanol) to the 12 ml Zyppy™ Wash Buffer concentrate (D4019), or 192 ml of 100% ethanol (208 ml of 95% ethanol) to the 48 ml Zyppy™ Wash Buffer concentrate (D4020 & D4037) before use.

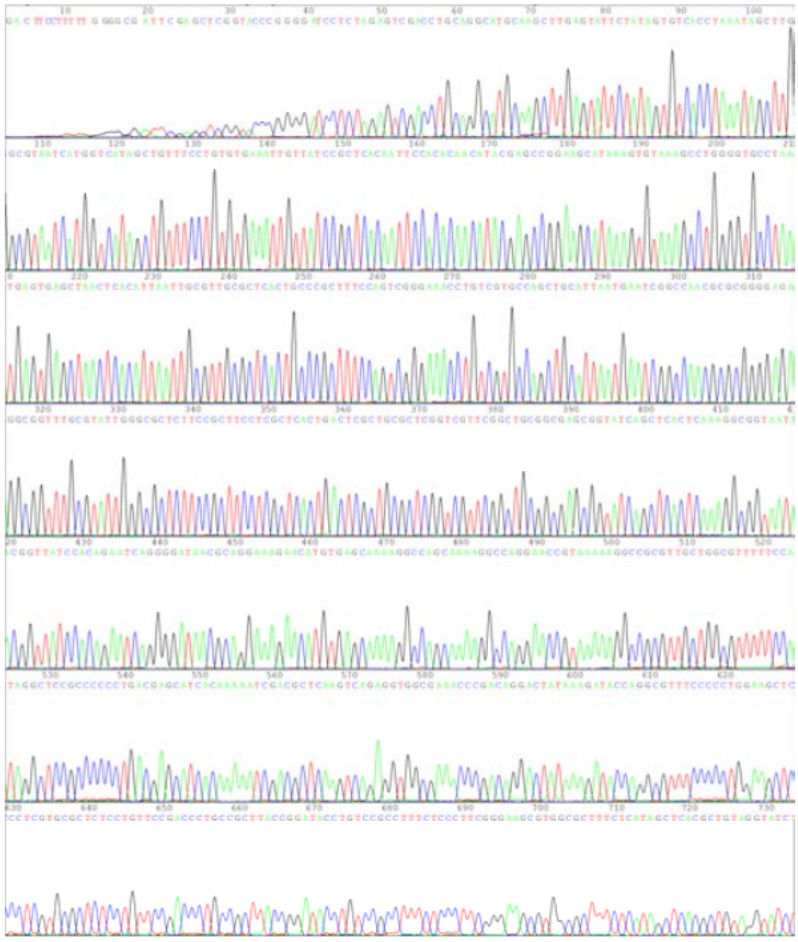
[4] Caution: 7X Lysis Buffer contains NaOH and Neutralization Buffer contains chaotropic reagents. Please use proper safety precautions with these reagents.

Product Description

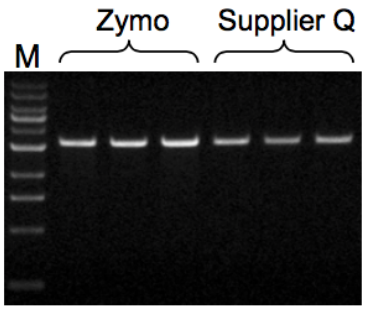
The **Zyppy™ Plasmid Miniprep Kit** features a **Pellet-Free™** modified alkaline lysis method that bypasses bacterial culture centrifugation and resuspension steps common to classical plasmid preparation procedures. Simply add the uniquely formulated **7X Lysis Buffer** directly to your bacterial culture, neutralize, then purify using our Fast-Spin column technology (alternatively, the samples may be processed by the classical centrifugation method). Additionally, the innovative colored buffers included in the kit permit error-free visualization identification of complete bacterial cell lysis and neutralization.

Our **Zyppy™ Plasmid Miniprep Kit** is the fastest and simplest method available to efficiently separate plasmid DNA from E. coli. The plasmid DNA is of the highest quality, is endotoxin-free, and is well suited for use in bacterial transformation, restriction endonuclease digestion (below right), DNA ligation, PCR, transcription, sequencing (below), and other sensitive downstream applications. An overview of the purification procedure is shown to the right.





Sequencing chromatogram of plasmid DNA pGEM® purified using the **Zyppy™ Plasmid Miniprep Kit**. The DNA was labeled with ABI BigDye v3.1 terminators, cleaned using the **ZR DNA Sequencing Clean-up Kit™ (D4050, D4051)** and sequenced on an ABI 3730*x*/ DNA analyzer.



*Eco*RI digestion of plasmid DNA (pGEM®) isolated from a 600 µl *E. coli* culture using the **Zyppy™ Plasmid Miniprep Kit** or a kit from Supplier Q. Performed in triplicate. M, ZR 1 kb DNA Marker.

Specifications

- **DNA Purity**: Plasmid DNA is well suited for ligation, sequencing, restriction endonuclease digestion, in vitro transcription, and other sensitive applications requiring pure DNA. Typical $Abs_{260}/_{280}$ index is ≥ 1.8.

- **Plasmid DNA Yield:** Up to 25 µg per preparation, depending on the plasmid copy number, culture growth conditions, and strain of E. coli utilized.

- **Plasmid DNA Size**: Up to 25 kb.

- **Recovery Volume**: ≥ 30 µl.

- **Procedure**: Performed at room temperature (15-30°C).

Buffer Preparation:

1. Add 24 ml of 100% ethanol (26 ml of 95% ethanol) to the 6 ml **Zyppy™ Wash Buffer** concentrate (D4036), 48 ml of 100% ethanol (52 ml of 95% ethanol) to the 12 ml **Zyppy™ Wash Buffer** concentrate (D4019), or 192 ml of 100% ethanol (208 ml of 95% ethanol) to the 48 ml **Zyppy™ Wash Buffer** concentrate (D4020 & D4037) before use.

2. The **7X Lysis Buffer** may have precipitated during shipping. To completely resuspend the buffer, incubate the bottle at 30 – 37 °C for 30 minutes and mix by inversion. DO NOT MICROWAVE.

Troubleshooting:

Please see the product PDF.

Before start

The procedure is performed at room temperature.

Buffer Preparation:

1. Add 24 ml of 100% ethanol (26 ml of 95% ethanol) to the 6 ml **Zyppy™ Wash Buffer** concentrate (D4036), 48 ml of 100% ethanol (52 ml of 95% ethanol) to the 12 ml **Zyppy™ Wash Buffer** concentrate (D4019), or 192 ml of 100% ethanol (208 ml of 95% ethanol) to the 48 ml **Zyppy™ Wash Buffer** concentrate (D4020 & D4037) before use.

2. The **7X Lysis Buffer** may have precipitated during shipping. To completely resuspend the buffer, incubate the bottle at 30 – 37 °C for 30 minutes and mix by inversion. DO NOT MICROWAVE.

Materials

Zyppy™ Plasmid Miniprep Kit D4036 D4019 D4020 D4037 by Zymo Research

Protocol

Step 1.
Add 600 µl of bacterial culture grown in LB medium to a 1.5 ml microcentrifuge tube.

NOTES
Shea Biondi 25 Nov 2016

The Zyppy™ Plasmid Miniprep Kit *may also be used with the classical centrifuge- based procedure for processing up to 3 ml of bacterial culture. The procedure should be modified as follows: 1A) Centrifuge 1.5 ml of bacterial culture for 30 seconds at maximum speed. 1B) Discard the supernatant. 1C) Repeat steps 1A and 1B as needed. 1D) Add 600 µl of TE or water to the bacterial cell pellet and resuspend completely.*

Step 2.
Add 100 µl of 7X Lysis Buffer (Blue)[1] and mix by inverting the tube 4-6 times. Proceed to step 3 within 2 minutes.

NOTES
Shea Biondi 25 Nov 2016

After addition of 7X Lysis Buffer the solution should change from opaque to clear blue, indicating complete lysis.

Shea Biondi 25 Nov 2016

[1] Excessive lysis can result in denatured plasmid DNA. If processing a large number of samples, we recommend working with groups of ten or less at a time. Continue with the next set of ten samples after the first set has been neutralized and mixed thoroughly.

Step 3.
Add 350 µl of cold Neutralization Buffer (Yellow) and mix thoroughly. *The sample will turn yellow when the neutralization is complete and a yellowish precipitate will form.*

Step 4.

Invert the sample an additional 2-3 times to ensure complete neutralization.

Step 5.
Centrifuge at 11,000 – 16,000 x g for 2-4 minutes.

DURATION
00:04:00

Step 6.

Transfer the supernatant (900 µl) into the provided **Zymo-Spin™ IIN** column. Avoid disturbing the cell debris pellet.

Step 7.
Place the column into a **Collection Tube** and centrifuge for 15 seconds.

DURATION
00:00:15

Step 8.
Discard the flow-through and place the column back into the same **Collection Tube.**

Step 9.
Add 200 µl of **Endo-Wash Buffer** to the column. Centrifuge for 30 seconds.

DURATION
00:00:30

NOTES
Shea Biondi 25 Nov 2016

It is not necessary to empty the collection tube.

Step 10.
Add 400 µl of **Zyppy™ Wash Buffer** to the column. Centrifuge for 1 minute.

DURATION
00:01:00

Step 11.

Transfer the column into a clean 1.5 ml microcentrifuge tube then add 30 µl of Zyppy™ Elution Buffer[2] directly to the column matrix and let stand for one minute at room temperature.

DURATION
00:01:00

NOTES
Shea Biondi 25 Nov 2016

[2] The Zyppy™ Elution Buffer contains 10 mM Tris-HCl, pH 8.5 and 0.1 mM EDTA. If required, pure water (neutral pH) can also be used to elute the DNA.

Step 12.

Centrifuge for 30 seconds to elute the plasmid DNA.

DURATION
00:00:30

Warnings

Caution: 7X Lysis Buffer contains NaOH and Neutralization Buffer contains chaotropic reagents. Please use proper safety precautions with these reagents.

Ensure that buffers have been prepared according to the instructions in the Guidelines.