Lambda DNA Purification Kit

INSTRUCTION MANUAL

Catalog #200391 and #200392

Revision B.0

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200391-12

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Lambda DNA Purification Kit

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Lambda DNA Purification Kit

MATERIALS PROVIDED

Materials provided	Catalog #200391	Catalog #200392	Storage conditions
80% DEAE-cellulose	25 ml	2 × 25 ml	4°C
DNase I (20 mg/ml)	50 µl	2 × 50 µl	–20°C
RNase A (2 mg/ml)	200 µl	2 × 200 µl	–20°C
Pronase (50 mg/ml)	770 µl	2 × 770 µl	–20°C
Glycogen (20 mg/ml)	75 µl	2 × 75 µl	–20°C
5% CTAB	1.5 ml	2 × 1.5 ml	Room temperature
0.5 M EDTA	2 ml	2 × 2 ml	Room temperature
1.2 M NaCl	10 ml	2 × 10 ml	Room temperature

STORAGE CONDITIONS

RNase: –20°C
DNase: –20°C
Glycogen: –20°C
Pronase: –20°C
DEAE Cellulose: 4°C
All Other Reagents: Room Temperature

ADDITIONAL MATERIALS REQUIRED

LB broth‡
SM buffer‡
NZY top agar‡
NZY agar plates‡
Chloroform
70% (v/v) Ethanol
100% (v/v) Ethanol
TE buffer‡
Maltose
Magnesium sulfate

‡ See *Preparation of Media and Reagents*

Revision B.0

INTRODUCTION

The Lambda DNA Purification Kit facilitates the rapid purification of high-grade lambda DNA eliminates the need for toxic phenol–chloroform extraction and time-consuming polyethylene glycol (PEG) precipitation steps used in traditional lambda miniprep procedures. The Lambda DNA Purification Kit uses diethylaminoethyl (DEAE) resin to remove contaminating polyanions prior to phage particle disruption.[1] The phage are lysed with ethylenediaminetetraacetic acid (EDTA) and pronase and the lambda DNA is then selectively precipitated with the cationic detergent cetyltrimethylammonium bromide (CTAB). Following an exchange reaction with sodium chloride, the highly purified lambda DNA is precipitated with ethanol. The Lambda DNA Purification Kit can be used for either liquid or plate lysates.[2]

PROTOCOL

Preparation of Plating Cultures

1. Inoculate 50 ml of LB broth containing 0.2% (v/v) maltose and 10 mM $MgSO_4$ in a sterile flask with a single colony of XL1-Blue cells or another appropriate bacterial host strain.

   Note *DO NOT add antibiotic to the overnight culture or to titering plates.*

2. Grow the culture overnight with shaking at 30°C. This temperature ensures that the cells will not overgrow. Phage can adhere to nonviable cells resulting in a decreased titer.

3. Spin down the cells in a sterile conical tube for 10 minutes at 2000 rpm.

4. Carefully decant the medium off the cell pellet and gently resuspend the pellet in ~15 ml of 10 mM $MgSO_4$ (do not vortex).

5. Dilute the cells to an OD_{600} of 0.5 with 10 mM $MgSO_4$.

6. The cells may be stored for 2–3 days at 4°C.

7. Proceed to step 1 of either *Preparation of Plate Lysate* or *Preparation of Liquid Lysate*.

Preparation of Plate Lysate

1. Mix 200 µl of the prepared host cells with at least 5000 pfu of lambda phage stock (100 µl) in a Falcon® 2059 tube.

2. Incubate the tube at 37°C for 20 minutes.

3. Add 3 ml of 48°C NZY top agar and spread on a 100-mm NZY agar plate. Allow the NZY top agar to harden at room temperature for 10 minutes.

4. Incubate the plate overnight at 37°C.

5. Overlay the plate with 3 ml of SM buffer.

6. Incubate the plate at room temperature for 3 hours or at 4°C overnight.

7. Remove and transfer the SM buffer (now containing lambda phage) to a fresh Falcon 2059 tube.

8. Add 1/50 volume of chloroform and vortex the tube.

9. Incubate the tube at room temperature for 10 minutes.

10. Titer to ensure $>5 \times 10^{10}$ pfu/ml.

11. Proceed to *Purification of Lambda DNA.*

Preparation of Liquid Lysate

1. Mix 100 µl of the prepared host cells with 1×10^6 pfu of lambda phage stock (100 µl) in a Falcon 2059 tube.

2. Incubate the at 37°C for 20 minutes.

3. Add 4 ml of LB broth to the tube.

4. Incubate the tube at 37°C with vigorous shaking for 5 hours until lysis occurs.

5. Add two drops of chloroform.

6. Incubate the tube at room temperature for 15 minutes with shaking.

7. Centrifuge the tube at 14,000 rpm for 10 minutes.

8. Remove and transfer the supernatant to a fresh Falcon 2059 tube.

9. Titer to ensure $>1 \times 10^{10}$ pfu/ml.

10. Proceed to *Purification of Lambda DNA.*

Purification of Lambda DNA

1. Remove any residual cell debris by centrifugation at 14,000 rpm for 10 minutes.

2. Place the supernatant in a fresh microcentrifuge tube if necessary (1 ml/tube).

3. Add 1 µl of DNase I (20 mg/ml) to the tube to a final concentration of 20 µg/ml.

   Note *Add 4 µl of RNase A (2 mg/ml) to the tube to a final concentration of 8 µg/ml. When using phage prepared from plate lysate, add RNase during step 12 of this protocol.*

4. Incubate the tube at room temperature for 15 minutes.

5. Microcentrifuge the tube at 14,000 rpm for 5 minutes at room temperature.

6. Transfer ~1 ml of the supernatant to a fresh tube. DO NOT CARRY OVER ANY PRECIPITATE.

7. Vigorously shake the tube containing the DEAE cellulose slurry to mix and then pipet 500 µl (~ 1/2 volume) of the DEAE slurry into the tube.

8. Incubate the tube at room temperature for 10 minutes, mixing the contents of the tube every 2 minutes by hand.

9. Microcentrifuge the tube for 1 minute at room temperature.

10. Place the supernatant in a fresh tube.

    Note *Resin will inhibit the modifying enzyme activity. Spin the tube again if the resin carries over.*

11. Add 40 µl of 0.5 M EDTA to the tube to a final concentration of 20 mM EDTA.

12. Add 15.4 µl of pronase stock solution (50 mg/ml) to the tube.

    Note *Add RNase at this step if using phage prepared from plate lysate.*

13. Incubate the tube at 37°C for 15 minutes.

14. Add 30 µl of 5% CTAB stock solution to the tube to a final concentration of 0.1% (v/v).

    Note *If CTAB precipitates out before use, place the CTAB at 37–65°C to drive the CTAB back into solution.*

15. Incubate the tube at 65°C for 3 minutes.

16. Add carrier glycogen to the tube to a final concentration of 20 µg/ml if phage titer is 1×10^{10} or lower to help precipitate the lambda DNA. (Add 1 µl of glycogen supplied with the kit per ml of reaction, ~1.5 µl.)

17. Incubate the samples on ice for 5 minutes to cool.

18. Microcentrifuge the samples at 14,000 rpm for 10 minutes.

19. Remove and discard the supernatant.

20. Gently resuspend the pellet in 200 µl of 1.2 M NaCl (1/5 volume from step 2 above).**DO NOT VORTEX.**

    Note *If the pellet does not dissolve in 10 minutes at room temperature or at 37°C, spin the sample in a microcentrifuge and transfer the supernatant, which contains the DNA, to a fresh microcentrifuge tube and continue with the next step.*

21. Add 500 µl of 100% (v/v) ethanol (2.5 volumes) and invert the tube to mix.

22. Microcentrifuge at 14,000 rpm for 10 minutes at room temperature. Remove and discard the supernatant.

23. Wash the pellet with 70% (v/v) ethanol. Repeat wash once more.

24. Dry the pellet and resuspend the lyophilized pellet in TE buffer.

    Note *The pellet will be spread on the side of the tube.*

Expected Yields

50% of the theoretical yield
1×10^{10} pfu = 0.25 µg lambda DNA from the liquid lysate method
5×10^{10} pfu = 1 µg lambda DNA from the plate lysate method
1×10^{11} pfu = 2 µg lambda DNA from the plate lysate method

TROUBLESHOOTING

Observation	Suggestion
Precipitation of the CTAB	CTAB will precipitate when stored at 4°C or below. Warm the CTAB before use.

PREPARATION OF MEDIA AND REAGENTS

DEAE-Cellulose Suspension Buffer

100 mM NaCl
10 mM Tris-HCl (pH 7.5)
50 mM $MgSO_4$

LB Broth (per Liter)

10 g of NaCl
10 g of bacto-tryptone
5 g of bacto-yeast extract
Adjust to pH 7.0 with 5 N NaOH
Add deionized H_2O to a final volume of 1 liter
Autoclave

SM Buffer (per Liter)

5.8 g of NaCl
2.0 g of $MgSO_4 \cdot 7H_2O$
50.0 ml of 1 M Tris-HCl (pH 7.5)
5.0 ml of 2% (w/v) gelatin
Add H_2O to a final volume of 1 liter
Autoclave

TE Buffer

10 mM Tris-HCl (pH 7.5)
1 mM EDTA

NZY Broth (per Liter)

5 g of NaCl
2 g of $MgSO_4 \cdot 7H_2O$
5 g of yeast extract
10 g of NZ amine (casein hydrolysate)
Adjust the pH to 7.5 with NaOH

NZY Top Agar (per Liter)

1 liter of NZY broth
Add 0.7% (w/v) agarose

NZY Agar (per Liter)

5 g of NaCl
2 g of $MgSO_4 \cdot 7H_2O$
5 g of yeast extract
10 g of NZ amine (casein hydrolysate)
15 g of agar
Adjust the pH to 7.5 with NaOH
Autoclave
Pour into petri dishes (~80 ml/150-mm plate)

REFERENCES

1. Manfioletti, G. and Schneider, C. (1988) *Nucleic Acids Res* 16(7):2873-84.
2. Cline, J., Troutman, M. and Mathur, E. (1992) *Strategies* 5(2):47.

ENDNOTES

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

STRATAGENE

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Lambda DNA Purification Kit

Catalog #200391 (50 1-ml minipreps) and #200392 (100 1-ml minipreps)

QUICK-REFERENCE PROTOCOL

- Prepare the plate or liquid lysate
- Remove the cell debris
- Treat with DNase I at room temperature for 15 minutes
    - Treat with RNase if using liquid lysate
- Spin and transfer the supernatant
- Treat with a DEAE slurry at room temperature for 10 minutes
- Spin and transfer the supernatant
- Add EDTA and treat with pronase at 37°C for 15 minutes
    - Treat with RNase if using plate lysate
- Treat with CTAB at 65°C for 3 minutes
- Add glycogen (only with the liquid lysate samples) and incubate the samples on ice for 5 minutes
- Spin and discard the supernatant
- Resuspend the pellet and precipitate with 100% (v/v) ethanol
- Wash the pellet with 70% (v/v) ethanol and resuspend the pellet in TE buffer