Modified Qiagen Plasmid Midi

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Abstract

This protocol is used for those that do not have a ≥20,000 x g capable centrifuge.

Citation: Michael Crone Modified Qiagen Plasmid Midi. **protocols.io**
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Before start

Add RNase A solution to Buffer P1, mix, and store at 2–8°C.
Optional: Add LyseBlue® reagent to Buffer P1 at a ratio of 1:1000.
Prechill Buffer P3 at 4°C. Check Buffer P2 for SDS precipitation.
Isopropanol and 70% ethanol are required.

Protocol

Step 1.
Harvest overnight bacterial culture by centrifuging at 4000 x g for 15 min at 4°C.

Lysis

Step 2.
Resuspend the bacterial pellet in 4 ml Buffer P1.

AMOUNT
4 ml Additional info:

REAGENTS
Buffer P1 by Contributed by users

Lysis

Step 3.
Add 4 ml Buffer P2, mix thoroughly by vigorously inverting 4–6 times, and incubate at room temperature (15–25°C) for 5 min. If using LyseBlue reagent, the solution will turn blue.

AMOUNT
4 ml Additional info:

REAGENTS
Buffer P2 by Contributed by users

DURATION
00:05:00

Lysis

Step 4.
Add 4 ml prechilled Buffer P3, mix thoroughly by vigorously inverting 4–6 times. Incubate on ice for 15 min. If using LyseBlue reagent, mix the solution until it is colorless.

AMOUNT

4 ml Additional info:

REAGENTS

Buffer P3 19053 by Contributed by users

DURATION

00:15:00

Lysis

Step 5.

Centrifuge at 14,000 x g for 45 min at 4°C.

DURATION

00:45:00

Equilibration

Step 6.

Equilibrate a QIAGEN-tip 100 by applying 4 ml Buffer QBT, and allow column to empty by gravity flow.

AMOUNT

4 ml Additional info:

REAGENTS

Buffer QBT by Contributed by users

Binding

Step 7.

Apply the supernatant from step 5 to the QIAGEN-tip and allow it to enter the resin by gravity flow.

Wash

Step 8.

Wash the QIAGEN-tip with 2 x 10 ml Buffer QC. Allow Buffer QC to move through the QIAGEN-tip by gravity flow.

AMOUNT

20 ml Additional info:

REAGENTS

Buffer QC by Contributed by users

Elution

Step 9.

Elute DNA with 5 ml Buffer QF into a clean 15 ml vessel. For constructs larger than 45 kb, prewarming the elution buffer to 65°C may help to increase the yield.

AMOUNT

5 ml Additional info:

REAGENTS

Buffer QF by Contributed by users

Precipitation

Step 10.

Precipitate DNA by adding 3.5 ml room-temperature isopropanol to the eluted DNA and mix. Centrifuge at 4,000 x g for 60 min at 4°C. Carefully decant the supernatant.

AMOUNT

4 ml Additional info:

DURATION

01:00:00

Precipitation

Step 11.

Wash the DNA pellet with 2 ml room-temperature 70% ethanol and centrifuge at 4,000 x g for 10 min. Carefully decant supernatant.

AMOUNT

2 ml Additional info:

REAGENTS

Ethanol BE-BDH1156 by P212121

DURATION

00:10:00

Resuspension

Step 12.

Air-dry pellet for 5–10 min and redissolve DNA in a suitable volume of appropriate buffer (e.g., TE buffer, pH 8.0, or 10 mM Tris·Cl, pH 8.5).

DURATION

00:10:00