SEP 07, 2023

OPEN ACCESS

Protocol Citation: Bettina Ergün 2023. E.Z.N.A.® Plasmid DNA Maxi Kit Centrifugation Protocol (modified). **protocols.io** https://protocols.io/view/e-z-n-a-plasmid-dna-maxi-kit-centrifugation-protoc-czpjx5kn

Protocol status: Working
We use this protocol and it's working

Created: Sep 07, 2023

Last Modified: Sep 07, 2023

PROTOCOL integer ID: 87499

E.Z.N.A.® Plasmid DNA Maxi Kit Centrifugation Protocol (modified)

Bettina Ergün[1]

[1]Charité

Urology_Research

Bettina Ergün

ABSTRACT

Describes a modified protocol to use the E.Z.N.A.® Plasmid DNA Maxi Kit # D6922 from Omega Bio-Tek without Ultracentrifuge.
For additionally information read the manufacturer's manual (attached)

ATTACHMENTS

D6922_E.Z.N.A. Plasmid DNA Midi Kitv4.2.pdf

MATERIALS

E.Z.N.A.® Plasmid DNA Maxi Kit # D6922 from Omega Bio-Tek
Centrifuge with swing bucket rotor capable of 4,000g
Nuclease-free 50 mL centrifuge tubes
100% ethanol (do not use denatured alcohol)
100% isopropanol
Optional: Water bath, incubator, or heat block capable of 65°C
Optional: Sterile deionized water
Optional: 3M NaOH

Before Start

1 Prepare DNA Wash Buffer, HBC Buffer, and Solution I:

- Add vial of RNase A to the bottle of Solution I provided and store at 2-8˚C.
- Dilute DNA Wash Buffer with 100% ethanol as writen on the bottle and store at room temperature.
- Dilute HBC Buffer with 100% isopropanol as writen on the bottle and store at room temperature.

2

- Check Solution II and Solution III for precipitation before use. Redissolve any precipitates by warming to 37°C
- Optional: Heat Elution Buffer to 65°C if plasmid DNA is >10 kb
- All centrifugation steps should be carried out at room temperature unless otherwise noted.

Samples

3 *place here the informations on the samples*

Extraction and purification

4 Transfer 50-200 mL overnight culture to an appropriate centrifuge tube

Note: The optimal volume to use depends on the culture density and plasmid copy number. The optimal cell mass (OD600 x mL culture) for the HiBind® DNA Maxi Column is 300-400. For example, if the OD600 of a culture is 4.0, the optimal culture volume should be 75-100 mL. If excess culture cell mass is used, alkaline lysis will be inefficient, the HiBind® membrane will be overloaded, and the performance of the system will be decreased. The increase in lysate viscosity will require vigorous mixing which may result in shearing of genomic DNA and contamination the plasmid DNA.

5 Centrifuge at 4,000g for 10 minutes.

6 Decant or aspirate and discard the culture media.

Note: To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the tube.

7 Add 12 mL Solution I/RNase A. Vortex or pipet up and down to completely resuspend the cells.

Note: RNase A must be added to Solution I before use.

8 Transfer the cell suspension to a 50 mL centrifuge tubes.

9 Add 12 mL Solution II. Invert and rotate the tube 2-3 minutes on a IKA rocker 3D at 25 rpm to obtain a cleared lysate.

Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution II tightly capped when not in use to avoid acidification from CO2 in the air.

10 Add 16 mL Solution III. Invert and rotate the tube 2-3 minutes on a IKA rocker 3D at 25 rpm until flocculent white precipitates form.

Note: It is vital that the solution is mixed thoroughly and immediately after the addition of Solution III to avoid localized precipitation.

11 Centrifuge at 4258g (max. g!) for 30 minutes at 4°C. Otherwise use a Ultracentrifuge at 15000g for 10 min (Original protocol) A compact white pellet will form. Promptly proceed to the next step.

12 Insert a HiBind® DNA Maxi Column into a 50 mL Collection Tube (provided).

12.1 Optional Protocol for Column Equilibration:

1. Add 3 mL 3M NaOH to the HiBind® DNA Maxi Column.
2. Let sit at room temperature for 4 minutes.
3. Centrifuge at 4,000g for 3 minutes.
4. Discard the filtrate and reuse the collection tube.

13 Transfer 20 mL cleared supernatant from Step 8 by CAREFULLY pipetting it into the HiBind® DNA Maxi Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Maxi Column.

14 Centrifuge at 4,000g for 5 minutes.

15 Discard the filtrate and reuse the collection tube.

16 Repeat Steps 10-12 until all of the cleared supernatant has been transferred to the HiBind® DNA Maxi Column.

17 Add 10 mL HBC Buffer.

Note: HBC Buffer must be diluted with 100% isopropanol prior to use. Please see the instructions in the “Preparing Reagents” section on Page 6.

18 Centrifuge at 4,000g for 5 minutes.

19 Discard the filtrate and reuse the collection tube.

20 Add 15 mL DNA Wash Buffer.
Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use.

21 Centrifuge at 4,000g for 5 minutes.

22 Discard the filtrate and reuse the collection tube.

23 Repeat Steps 20-22 for a second DNA Wash Buffer wash step.

24 Centrifuge the empty HiBind® DNA Maxi Column at 4,000g for 10 minutes to dry the column matrix.

Note: It is important to dry the HiBind® DNA Maxi Column matrix before elution. Residual ethanol may interfere with downstream applications.

25 Transfer the HiBind® DNA Maxi Column to a nuclease-free 50 mL centrifuge tube (not provided).

26 Add 1.5-3 mL Elution Buffer or sterile deionized water directly to the center of the column membrane.

27 Let it sit at room temperature for 5 minutes.

Note: This represents approximately 65-80% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration. Alternatively, a second elution may be performed using the first eluate to maintain a high DNA concentration.

28 Use the first eluat for a second elution step.

QC and storage

29 Qubit Concentration determination as described in separate protocol.

29.1 *place here the concentrations and the aliquotation of the samples*

30 Aliquot and store DNA at -20°C.