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Plasmid Extraction

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

This protocol is used for extracting plasmid from E. coli DH5-Alpha strains.

PROTOCOL CITATION

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GUIDELINES

The manual from manufacturer is provided here.

Presto™ Mini Plasmid Kit.pdf

This protocol is usually performed after overnight incubation of bacteria in a centrifuge tube.

MATERIALS

NAME	CATALOG #	VENDOR
Presto™ Mini Plasmid Kit (PDH100 PDH300)	PDH100	Geneaid

ABSTRACT

This protocol is used for extracting plasmid from E. coli DH5-Alpha strains.

BEFORE STARTING

Follow the "preparation" section of protocol.

Preparation

1 Set the dry bath incubator at **37 °C**

2 Prepare Presto™ Mini Plasmid Kit

3 Take out the centrifuge tube containing medium with bacteria that has been cultured overnight.

Protocol

4 Centrifuge the centrifuge tube under this condition - **15000 x g, 00:06:00** .

5 Discard the supernatent

Recommendation: Keep the bacterial pellet away from supernatent if plausible to insure minimum loss of bacteria.

6 Add **200 µl** of PD1 reagent to the centrifuge tube. Vortex the tube to completely mix bacterial pellet with PD1.

7 Add **200 µl** of PD2 reagent to the centrifuge tube. Shake the tube gently and let it stand for **00:02:00** to **00:05:00** .

Warning: Do not vortex the mixture after adding PD2!!!!

8 Add **300 µl** of PD3 reagent to the centrifuge tube. Shake the tube gently and centrifuge the tube under this condition - **16000 x g, 00:10:00** .

Warning: Do not vortex the mixture after adding PD3!!!!
Tips: Prepare the PDH column when centrifuging.

9 After centrifugation, transfer the supernatent into a PDH column and discard the pellet.
Centrifuge the PDH column under **15000 x g, 00:00:30** and discard the flowthrough liquid in the collection tube.

10 Add **400 µl** of Wash 1 Buffer into the PDH column and centrifuge under **15000 x g, 00:00:30** .
Discard the flowthrough in the collection tube.

11 Add **600 µl** of Wash 2 Buffer into the PDH column and centrifuge under **15000 x g, 00:00:30** .
Discard the flowthrough in the collection tube.

12 Centrifuge again under **15000 x g, 00:03:00** and replace the collection tube with a new eppendorf. (Discard the flowthrough)

13 Add **50 µl** of Elution Buffer into the middle of PDH column and incubate the PDH column in a drybath incubater under **37 °C** .

Recommendation: Add the Elution Buffer directly onto the silicon membrane to insure optimal euting efficacy.

14 Centrifuge under **15000 x g, 00:02:00** . The flowthrough in the eppendorf contains plasmids derived from the cultured bacteria.

Recommedation: Proceed to measure the concentration of plasmid.