Plasmid DNA isolation (NC) kit

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DENA ZIST ASIA
MOLECULAR BIOLOGY PRODUCTS
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Manual

Description: DENAzist plasmid DNA isolation NC kit is devised for simple and efficient isolation of plasmid DNA based on alkaline lysis of bacterial cells which have amplified their plasmid copies during culture. The plasmid DNA extracted by this kit can be used in restriction enzyme digestion, PCR amplification reactions, and cloning projects. RNase treatment of the samples at the beginning of the isolation process removes RNA contaminations from the final product.

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PROTOCOL for isolation of plasmid DNA using DENAzist plasmid isolation NC kit

Before starting, make sure that you have prepared 95% and 70% ethanol. If precipitates are present in NC2 buffer, warm it in a 37 °C incubator to get all the buffer into the solution.

1. Spin down 3 ml of overnight bacterial culture (with high-copy number plasmid) or 5-10 ml culture (with low-copy number plasmid) in a 1.5 ml microcentrifuge tube by centrifugation at 13,000 rpm for 1 min. After each centrifugation, discard the supernatant, keep the pellet, and add more from the culture for another round of centrifuge. Keep the pellet of bacteria at the end of this stage.
2. By pipetting and vortexing, completely and thoroughly resuspend the bacterial pellet in 100 µl of **NC1** buffer. Add 10 µl **RNase A** to the tube. Incubate the tube at room temperature for 5 min.
3. Add 200 µl from **NC2** buffer. Mix the contents by inverting the tube several times. Incubate the tube on ice for 5 min.
4. Add 150 µl from **NC3** buffer. Mix by inverting the tube several times. Incubate the tube on ice for 5 min.
5. Centrifuge the tube for 10 min at 13,000 rpm in a table-top microcentrifuge. A compact white pellet will form at the bottom of tube.
6. Transfer the supernatant (cleared lysate) from the previous step to a new tube. Discard the pellet.
7. Add 800 µl from 95% ethanol to the tube. Mix and incubate the tube at room temperature for 3 min.
8. Centrifuge the tube at 13,000 rpm for 1 min. A pellet will form. Keep the pellet and discard the supernatant.
9. Add 1000 µl from 70% ethanol to the pellet. Briefly vortex the tube. Centrifuge the spin column at 10,000 rpm for 1 min. Discard the supernatant and keep the pellet.
10. Let the pellet to dry at room temperature by leaving open the cap of the tube for 10 minutes. Alternatively, the pellet can be dried under the vacuum.
11. Add 30-50 µl from **NC4** buffer and dissolve the pellet. Until further downstream processes store the sample in -20 °C freezer.◢

Kit components:

Buffers: NC1, NC2, NC3, NC4
Enzyme: RNase A

Store all components at room temperature except RNase A which should be stored at -20 °C.

Notes: Research Use Only
This product insert declares that this products has been analyzed and passed the quality control tests at the time of manufacture.