# Plasmid DNA Isolation Kit

Cat. No.: S-1040-5; 5 applications
Cat. No.: S-1040; 25 applications
Cat. No.: S-1040-1; 50 applications
Cat. No.: S-1040-250; 250 applications

DENA ZIST ASIA
MOLECULAR BIOLOGY PRODUCTS

## Manual

**Description:** DENAzist Plasmid DNA Isolation Kit is devised for the simple and quick isolation of plasmid DNA. By using this kit, depending on the culture conditions and the copy number of plasmid, pure plasmid DNA with a concentration of 500 to 1000 ng/µl can be isolated from 1.5 ml of bacterial culture. The high quality and purity of the extracted plasmid DNA allow its direct use in all kinds of downstream processes. In addition, RNase treatment during the isolation process removes RNA contaminations from the final product.

VERSION: Dec. 2022

### Important instructions before first use

For the 5 application kit, add 2.4 ml ethanol (96%-100%) to the container labeled DP4.
For the 25 application kit, add 12 ml ethanol (96%-100%) to the container labeled DP4.
For the 50 application kit, add 24 ml ethanol (96%-100%) to the container labeled DP4.
For the 250 application kit, add 60 ml ethanol (96%-100%) to each of the two containers labeled DP4.

## PROTOCOL for isolation of plasmid DNA

Before first use, make sure that ethanol has been added to the container labeled DP4.

1. Spin down 1.5 ml of overnight (14 hours) bacterial culture (with high-copy number plasmid) or 5 ml (with low-copy number plasmid) in a 1.5 ml microcentrifuge tube by centrifugation at 13,000 rpm for 1 min. Discard the supernatant and keep the pellet.
2. By pipetting, completely and thoroughly resuspend the bacterial pellet in 250 µl of **DP1**. The color of the solution turns cloudy light-red or pink.
3. Add 250 µl from the **DP2** solution. Mix the contents by gently inverting the tube several times until cell suspension buffer becomes clear red or pink. Incubate the tube at room temperature for 3 min.
4. Add 350 µl from **DP3**. Mix by gently inverting the tube several times. Color becomes yellow. Centrifuge the tube for 10 min at 13,000 rpm in a table-top microcentrifuge. A compact milky white pellet will form at the bottom of the tube. If the supernatant looks cloudy (not clear), transfer it to a new tube and repeat centrifugation.
5. While avoiding the pellet, transfer 750 µl from the supernatant (cleared lysate) into a 1.5 ml microcentrifuge tube. Add 750 µl from 96-100% ethanol and mix.

   ***Important:* during the transfer of supernatant (cleared lysate) to the tube, avoid pipetting the white pellet at the bottom of the tube. These precipitates can clog the spin column.**

-------DNA BINDING TO THE COLUMN------

6. Mount a spin column onto a collection tube (both are included in the kit). Using a sampler (pipettor), transfer 750 µl of the mix from step 5 to the upper reservoir of the spin column. Centrifuge the spin column at 10,000 rpm for 2 min. Discard the flow-through accumulated in the collection tube. Re-mount the spin column onto the collection tube.

   ***Important:* make sure that whole lysate has been thoroughly passed through the column and no visible amounts of lysate are present in the upper reservoir.**

7. Transfer the remaining 750 µl of the mix from step 5 to the spin column and repeat step 6.
8. In a microcentrifuge tube, mix 5 ul **RNase A** and 25 ul water. Add the mixture onto the center of spin column. Leave the spin column at room temperature for 5 to 15 min.

-------WASHING------

9. Add 700 µl from **DP4** solution into the spin column. Centrifugate the spin column at 10,000 rpm for 1 min. Discard the flow-through.
10. Without adding any solutions, centrifuge the spin column one more time at top speed (13,000 rpm) for 3 min.

-------ELUTION-------

11. Separate the spin column from its collecting tube and place it into a new 1.5 ml microcentrifuge tube.
12. Add 50-100 µl from **DP5** solution (or sterile water in case the plasmid will be used for transfection) onto the center of the spin column. Leave the spin column and its tube for 3 min at room temperature. Centrifuge the spin column and its tube at 13,000 rpm for 3 min.
13. Store the flow-through solution containing isolated plasmids in a -20 °C freezer. ◢

| Kit components | 5 app. | 25 app. | 50 app. | 250 app. |
|---|---|---|---|---|
| DP1 | 1.5 ml | 7.5 ml | 15 ml | 75 ml |
| DP2 | 1.5 ml | 7.5 ml | 15 ml | 75 ml |
| DP3 | 1.8 ml | 9 ml | 18 ml | 90 ml |
| DP4 | 1.8 ml | 9 ml | 18 ml | 90 ml |
| DP5 | 0.6 ml | 3 ml | 6 ml | 30 ml |
| RNase A | 28 µl | 138 µl | 275 µl | 1375 µl |
| Spin column | 5 | 25 | 50 | 250 |

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.