Circulating DNA isolation kit

Cat. No.: S-1036-5; 5 applications Cat. No.: S-1036; 25 applications Cat. No.: S-1036-1; 50 applications

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MANUAL

Description: DENAzist circulating DNA isolation kit is designed for the extraction of genomic DNA from plasma or serum. Using this kit, it is possible to extract genomic DNA from plasma or serum with the highest quality in the least amount of time. The provided protocol is designed for isolation of DNA from 1 ml plasma/serum.

VERSION: Sept 2023

Important instructions before first use

For the 5 application kit, add 2.4 ml ethanol (96%-100%) to the container labeled CD3. For the 25 application kit, add 12 ml ethanol (96%-100%) to the container labeled CD3. For the 50 application kit, add 24 ml ethanol (96%-100%) to the container labeled CD3. Label the container to record that ethanol has been added before first use.

PROTOCOL for isolation of genomic DNA from blood serum or plasma

Before first use, make sure that ethanol has been added to the container labeled CD3.

- 1. Centrifuge 1 ml of blood serum or plasma at 13,000-14,000 rpm for 10 min to pellet out any cellular material. Carefully transfer the supernatant to a 2 ml microfuge tube.
- 2. Add 15 µl **proteinase K** to the tube. Vortex for 10 seconds.
- 3. Add 50 µl CD1. Vortex for 10 seconds.
- 4. Add 1 ml CD2 to the tube and vortex for 5 seconds.
- 5. Incubate the tube at 60 °C for 30 minutes. Centrifuge the tube at 14,000 rpm for 5 min.
- 6. Transfer the supernatant from the previous step to a Falcon tube. Add 500 $\,\mu l$ ethanol. Thoroughly mix the contents.

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

- 7. Transfer 750 μ l of the mix into a spin column (inserted into a collection tube, both provided in the kit). Centrifuge the spin column at 10,000 rpm for 2 min. Discard the flow-through accumulated in the collection tube. Remount the spin column onto the collection tube.
- 8. Transfer the rest of the mix by repeating the step 7 several times.

-----WASHING-----

- 9. Add 700 µl from the **CD3** solution into the spin column. Centrifuge the spin column at 10,000 rpm for 1 min. Discard the flow-through.
- 10. Without adding any solutions, centrifuge the tube one more time at top speed (13,000 rpm) for 3 min.

11. Separate the spin column from its collecting tube and place it into a new 1.5 ml microfuge (Eppendorf) tube.

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

- 12. Add 50 μl from the CD4 solution onto the center of spin column. Leave the spin column mounted on the microfuge tube for 2 min at room temperature. Centrifuge the spin column mounted on the microfuge tube at 10,000 rpm for 2 min.
- 13. Return the eluted solution from the previous step back onto the center of the spin column. Leave the spin column mounted on the microfuge tube for 2 min at room temperature. Centrifuge the spin column mounted on the microfuge tube at 13,000 rpm for 3 min.
- 14. The eluted solution at the bottom of the microfuge tube contains pure genomic DNA. Until further downstream processes store the sample in -20 °C freezer. ▲

Kit components:			
	5 app.	25 app.	50 app.
CD1	1 ml	3 ml	6 ml
CD2	5 ml	25 ml	50 ml
CD3	1.8 ml	9 ml	18 ml
CD4	1 ml	3 ml	6 ml
Proteinase K	85 µl	390 µl	780 µl
Spin columns	5	25	50

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

Expiry date: 18 month after shipping

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.