

Manual Protocol for Oragene•DNA Purification using a Deep 96 -well Plate

Materials:

1. Plates and covers - deep 96-well (round) Plates (Axygen Cat No. P-DW-20-C) with 96-well reusable Mat or Adhesive Cover Sheet (Axygen Cat No. AM-2ML-RD-IMP).
2. Centrifuge with bucket to accommodate 96-well plates. Speed of at least 3,500 rpm required. (e.g., Sorvall centrifuge model RT 6000D with PN 11093 96-well plate adapter).
3. Purifier-Oragene Level 2.
4. Blue Dextran (Sigma-Aldrich Cat No. D5751).
5. 95% ethanol (EtOH) (room temperature).
6. 70% ethanol (EtOH) (room temperature).
7. TE Buffer (10 mM tris pH 8.0, 1 mM EDTA).
8. 8- or 12-channel multipipettor.

Method:

1. Add 20 μ L of Purifier to each well of the Plate.
2. Transfer 5 μ L of Dextran 1 mg/mL to each well of the Plate.
3. Transfer 500 μ L of Oragene•DNA sample to each well.
4. Cover Plate with Adhesive Cover Sheet or reusable mat. Press into place to seal. Mix manually by inversion 5 times.
5. Incubate at -20°C for 10 min.
6. Centrifuge Plate for 10 min, room temperature at 4,200 xg.
7. While first Plate is centrifuging, add 315 μ L of Isopropanol (room temp) to each well of a second 96- well Plate.
8. When centrifuge has stopped, transfer 450 μ L of the supernatant from the first Plate to the second Plate (containing Isopropanol). Take care not to disrupt the pellet.
9. Cover Plate with Adhesive Cover Sheet or reusable Mat. Press into place to seal. Mix manually by slowly inverting 10 times. DNA clot will form. Incubate at room temp for 10 minutes.
10. Centrifuge Plate for 10 min, room temperature at 4,200 xg.
11. Carefully remove as much supernatant as possible from each well without disturbing the pellet. Discard the supernatant. This step should be done with a single-tip pipette to avoid disturbing the pellet. Some pellets may be stuck to the side of the well, take care not to remove them with the pipette.
12. Add 400 μ L of 70% EtOH (room temp) to each well.
13. Cover Plate with Adhesive Cover Sheet or reusable Mat. Press into place to seal. Mix by vigorous vortexing.
14. Centrifuge Plate for 10 min, room temperature at 4,200 xg.
15. Carefully remove **ALL** the supernatant from each well without disturbing the pellet. Discard the supernatant. This step should be done with a single-tip pipette to avoid disturbing the pellet.
16. The Plate should be pulsed centrifuged for 20 seconds to collect any left over EtOH.
17. Using a pipette carefully remove **ALL** residual EtOH.
18. Air-dry the plate for 5 minutes. Dissolve the precipitated DNA in 50-100 μ L of TE. Vortex vigorously to ensure any pellet on the side of the well is dislodged and re-hydrated. Place the plate on a shaker or rocker to help fully re-hydrate the DNA pellet – at least 30 min. As per our manual protocol, allowing a longer time and higher temperature (37 - 50°C) will be beneficial.
19. Once dissolved, the DNA is ready for use or storage.