DNA extraction protocol (QIAGEN DNeasy96)

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Aims

Extract DNA from pellets in 96-well plates.

Materials

- PBS 36 mL
- proteinase K 2.5 mL
- buffer AL 20 mL
- ethanol 20 mL
- buffer AW1 50 mL
- buffer AW2 50 mL
- buffer AE 10 mL
- DNeasy96 plate 1
- S-block or DW96 1
- PCR plate 1

Procedures

☐ Thaw pellet plates at room temperature.
☐ Dissolve pellets in 180μL of PBS.
☐ Transfer the dissolved pellets to DNeasy microtube plates.
☐ Add 25μL proteinase K and 200μL Buffer AL.
☐ Seal the plate with caps and mix by vortexing. Incubate at **56C** for **30 min**.
☐ Add 200μL pure ethanol.
☐ Seal the plate with caps and mix thoroughly by vortexing.
☐ Assemble the DNeasy 96 plates on top of DW96s. Mark the DNeasy 96 plates.
☐ Use mP1000 set at 625μL to transfer the lysate of each sample from DW96s to each well of DNeasy96 plates (maximum 900μL).
☐ Seal DNeasy96 with AirPore film. Use vacuum to collect the flow-through.
☐ Add 500μL of Buffer AW1 to each sample.
☐ Seal DNeasy96 with AirPore film. Use vacuum to collect the flow-through.
☐ Add 500μL of Buffer AW2 to each sample.
☐ Seal DNeasy96 with AirPore film. Use vacuum to collect the flow-through.
☐ Label two PCR plates. Place DNeasy96s on these PCR plates.
☐ Add 100μL Buffer AE to each sample, and seal the DNeasy 96 plates with new AirPore Tape Sheets (provided).
☐ Incubate for 1 min at room temperature (15–25°C). Centrifuge for **2 min** at **6000 rpm**.
☐ Cover the PCR plates with aluminum foil and save it in -20C freezer.