DNEasy DNA Extraction - Vibrio Gram Negative Broth Bacteria

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Abstract

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Protocol

Step 1.
Put Buffer ATL and AL at 56 C for 5 minutes before using.

DURATION
00:05:00

Step 2.

Transfer 250ul of broth culture to 2ml deep well (autoclaved)

AMOUNT
250 µl Additional info:

Step 3.

Harvest cells (maximum 2 x 109 cells) by centrifuging plate for 15 min at 4063 x g. Discard supernatant.

DURATION
00:15:00

Step 4.
Resuspend pellet in 200 µl Buffer ATL by pipetting up and down 15 times.

AMOUNT
200 µl Additional info:

REAGENTS
Buffer ATL (tissue lysis buffer) 19076 by Qiagen

Step 5.
Seal with foil and place in oven at 56 C for one hour. Place extra plate on seal so foil doesn't come off.

DURATION
01:00:00

Step 6.
Pipette up and down 15 times and spin down. (May place in -80 C and continue procedure later at this point.)

Step 7.

If placed in -80 C, let thaw and spin down @ 1000g for 1 min

Step 8.
Add 205 uL Buffer AL and 205 uL molecular grade EtOH.

AMOUNT
205 µl Additional info:

REAGENTS
Buffer AL (lysis buffer) by Qiagen

Step 9.
Pipette up and down 15 times and spin down.

Step 10.

Place DNeasy 96 plates on top of 2ml deep well plate . Mark the DNeasy 96 plates for later sample identification.

Step 11.

Carefully transfer the lysate (approximately 600ul) of each sample from step 7 to each well of the DNeasy 96 plates. Do not transfer more than 900 µl per well.

Step 12.
Seal each DNeasy 96 plate with a porous film. Centrifuge for 15 min at 4063g. If lysate remains in any of the wells, centrifuge for a further 10 min.

DURATION
00:15:00

Step 13.
Add 500 µl Buffer AW1 to each sample.

AMOUNT
500 µl Additional info:

REAGENTS
Buffer AW1 19081 by Qiagen

Step 14.
Seal each DNeasy 96 plate with a new AirPore Tape Sheet (provided). Centrifuge for 5 min at 4063g.

DURATION
00:05:00

Step 15.
Remove the tape. Carefully add 500 µl Buffer AW2 to each sample. Centrifuge for 15 min at 4063g. Do not seal the plate with AirPore Tape. The heat generated during centrifugation ensures evaporation of residual ethanol in the sample (from Buffer AW2) that might otherwise inhibit downstream reactions.

AMOUNT
500 µl Additional info:

REAGENTS
Buffer AW2 19072 by Qiagen

DURATION
00:15:00

Step 16.

Place in new collection rack (2ml deep well plate) and spin again 15 minutes at 4063g.

DURATION
00:15:00

Step 17.

Place each DNeasy 96 plate in the correct orientation on a new rack of VWR 500ul plate.

Step 18.

To elute the DNA, add 200 µl Buffer TE to each sample, seal and incubate for 1 minute at room temp. Centrifuge for 4 min at 4063g. 200 µl Buffer TE is sufficient to elute up to 75% of the DNA from each well of the DNeasy 96 plate.

AMOUNT
200 µl Additional info:

REAGENTS
Buffer TE 1x by Contributed by users

DURATION
00:04:00

Step 19.

Recommended: For maximum DNA yield, repeat step 16 with another 200 µl Buffer TE.

AMOUNT
200 µl Additional info:

REAGENTS
Buffer TE 1x by Contributed by users

DURATION
00:04:00

Step 20.

Aliquot 20ul of extracted DNA to green 96 microplate. Seal DNeasy 96 plate and 96 microplate with non-sterile foil. Place DNeasy 96 plate in -80 C and 96 microplate in -20 C.

AMOUNT
20 µl Additional info: