General tube bacteria extraction (DNeasy)

Sarah Hessen-Schmidt

Abstract

Citation: Sarah Hessen-Schmidt General tube bacteria extraction (DNeasy). **protocols.io** dx.doi.org/10.17504/protocols.io.fbcbiiw
Published: 13 Jul 2016

Protocol

Step 1.

Place Buffer ATL and Buffer Al at 56C for at least 5 minutes before starting. Make sure AW1 and AW2 have ethanol added

Step 2.

Spin >500ul of bacteria liquid culture at 10000g for 10 minutes. Pour off supernatant

Step 3.

Add 200ul buffer ATL to each sample and vortex for 15s

AMOUNT
200 µl Additional info:
REAGENTS
Buffer ATL (tissue lysis buffer) 19076 by Qiagen
DURATION
00:00:15

Step 4.

Add 200ul 100% ethanol to each sample and vortex for 15s

AMOUNT
200 µl Additional info:
REAGENTS
Ethanol by Contributed by users
DURATION
00:00:15

Step 5.

Briefly spin to collect all liquid. Transfer all of sample including precipitate to spin column.

Step 6.

Centrifuge at max speed (>20000g) for 2 minutes

DURATION
00:02:00

Step 7.

Empty collection tube

Step 8.

Add 500ul buffer AW1 to spin column. Spin at max speed (>20000g) for 2 minutes

AMOUNT
500 µl Additional info:

REAGENTS
Buffer AW1 19081 by Qiagen

DURATION
00:02:00

Step 9.

Add 500ul buffer AW2 to spin column. Spin at max speed (>20000g) for 2 minutes

AMOUNT
500 µl Additional info:

REAGENTS
Buffer AW2 19072 by Qiagen

DURATION
00:02:00

Step 10.

Place spin column in a new collection tube and spin at max speed (>20000g) for 3 minutes

DURATION
00:03:00

Step 11.

Transfer spin column to a labeled 1.5ml Eppendorf lobind tube.

Step 12.

Add 100ul of buffer TE directly to membrane/filter in spin column and incubate at room temp. for 1 min

AMOUNT
100 µl Additional info:

REAGENTS

Buffer TE 1x by Contributed by users

DURATION

00:01:00

Step 13.

Spin at 10000g for 2 minutes

DURATION

00:02:00