Apr 27, 2018

Antibiotics gradient assay for V. natriegens

DOI
dx.doi.org/10.17504/protocols.io.ps8dnhw

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DOI: https://dx.doi.org/10.17504/protocols.io.ps8dnhw

Protocol Citation: Franziska Müller, Memduha Muratoglu 2018. Antibiotics gradient assay for V. natriegens. **protocols.io https://dx.doi.org/10.17504/protocols.io.ps8dnhw**

Protocol status: Working
We use this protocol and it's working

Created: April 27, 2018

Last Modified: April 27, 2018

Protocol Integer ID: 11840

Keywords: antibiotic, gradient assay

Troubleshooting

1 Inoculate preculture of *V. natriegens* in LB3 medium (3% NaCl) and incubate overnight at 37°C, shaking.

Preparation of gradient plates

2 Preparation of the first layer:
Place 11.5×11.5 cm plates in an inclient position. The angle of inclination is such that the agar layer deminishes to nothing at one edge of the plate. Pour 30 ml of LB3 with chosen antibiotics of concentrations to be tested into the plate. Let the agar solidify.

3 Switch position of the plate to an even surface and pour 30 ml of LB3 agar **without** any antibiotics to onto the first layer. Let the agar solidify.

Spotting of V. natriegens

4 Diltute the preculture to an OD550 of 0.1.

5 Pipette 8-10 spots of 5 µl of the culture along the gradient.

6 Let the spots dry and incubate the plates upside-down at 37°C over night or at room temperature over the weekend.

Measurement

7 Measure the distance from the edge of the agar plate to the last point of growth.

8 Calculate the highest concentration were *V. natriegens* can survive by using the following equation:
$c_{antibioticH} = c_{antibioticmax} * d_{growth}/d_{plate}$

$c_{antibioticH}$: highest antibiotic concentration *V. natriegens* can survive
$c_{antibioticmax}$: maximum atibiotics concentration (concentration used for the lower layer of the plate)
d_{growth}: distance from the edge of the agarplate to the last point of growth
d_{plate}: length of the plate (11.5 cm)