Protocol for Bacterial Heterotrophic Production

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Abstract

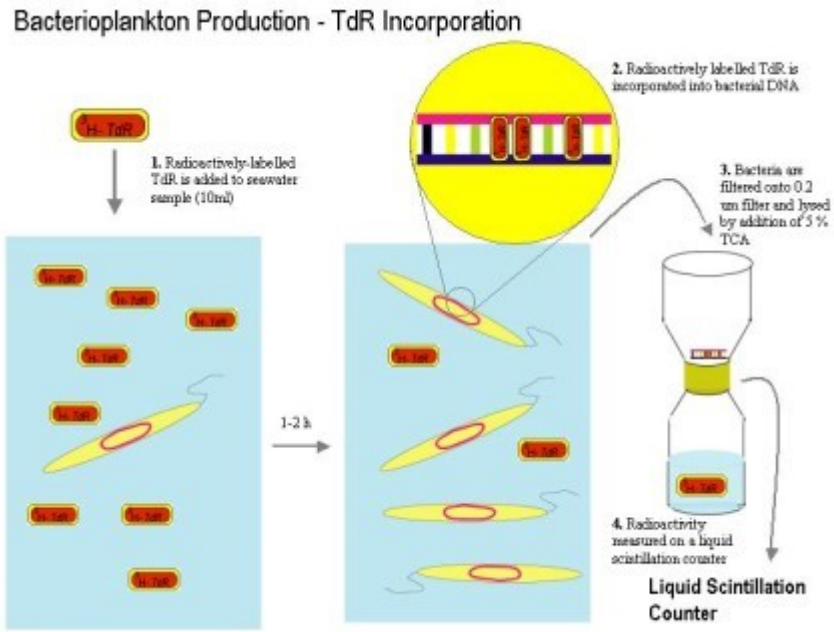
Citation: Jed Fuhrman Protocol for Bacterial Heterotrophic Production. **protocols.io**
dx.doi.org/10.17504/protocols.io.dmd425
Published: 14 Aug 2015

Guidelines

Materials:

- 10 place filter manifold
- Towers (chilled overnight @ 4C)
- 5% (w/v) Trichloroacetic acid (TCA) (chilled overnight @ 4C) Usually 1L is enough for a single time series set
- 32 15ml centrifuge tubes
- Pump
- Scintillation vials and fluid (UltimaGold)
- Lab supplies

Map:



Protocol

Procedure

Step 1.
Label in duplicate, **4** 15ml centrifuge tubes for each depth (3 samples and 1 control)

NOTES
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One set for Thymidine and another for Leucine.

Procedure
Step 2.
Rinse each tube 3x with 5ml of sample seawater

Procedure
Step 3.
Pour 10ml of sample into rinsed centrifuge tube

Procedure
Step 4.
Kill control samples by adding 0.1ml of filtered formalin

Procedure
Step 5.
Let stand 10 minutes

DURATION
00:10:00

Procedure
Step 6.
Pipette 5nM of tritiated Thymine or 5nM of tritiated Leucine into sample

NOTES
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Typically vol. TdR<vol Leu.

Procedure
Step 7.
Dispose of tips into labeled plastic whirlpak bags

Procedure
Step 8.
Incubate samples at seawater temperature for 1 hour

DURATION
01:00:00

Procedure
Step 9.
During incubation, label scintillation vials

Procedure
Step 10.
10 minutes before incubation time ends set-up Hoeffer box with 0.45µm HA Millipore filters

Procedure
Step 11.
Place chilled towers

Procedure
Step 12.
Following incubation pour samples into respective wells and filter through (<0.3 atm)

NOTES

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Make sure no leaks!!

Procedure

Step 13.

Turn off pump and close wells

Procedure

Step 14.

Add 2ml of 5% TCA to each well

Procedure

Step 15.

Let stand for 2 minutes

DURATION

00:02:00

Procedure

Step 16.

After 2 minutes, open wells and filter through TCA

Procedure

Step 17.

Rinse towers 3x with 5% TCA

Procedure

Step 18.

Remove towers (pump is still on!)

Procedure

Step 19.

Rinse filter 3x with 5% TCA, enough to cover filters completely

Procedure

Step 20.

Turn off pump

Procedure

Step 21.

Remove filters and place in labelled scintillation vials

Procedure

Step 22.

Add 5ml of UltimaGold scintillation fluid

Procedure

Step 23.

Tightly cap scint vial and shake HARD; make sure the filter ends up in the liquid

Procedure

Step 24.

Let sit for at least 3 hours before final counts, preferably overnight

DURATION

03:00:00

Procedure

Step 25.

Perform wipetest of surrounding area (see map in guidelines)

Procedure

Step 26.

Read samples in scintillation counter

NOTES

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Currently using program #1 (1 minute read).

Clean up

Step 27.

Rinse towers with diH_2O

Clean up

Step 28.

Rinse top of Hoeffer box with diH_2O

Clean up

Step 29.

Pour radioactive filtrate out of Hoeffer box into waste container carefully

NOTES

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Use a funnel!

Clean up

Step 30.

Throw dry waste into radioactive waste barrel

NOTES

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Not the regular trash!

Clean up

Step 31.

Record isotope usage online