Bacterial abundance from grass litter - Flow cytometry

cweihe [1]

[1]Jennifer Martiny Lab UC Irvine

Jun 11, 2021

1 Works for me Share dx.doi.org/10.17504/protocols.io.buznnx5e

Jennifer Martiny Lab

cweihe

ABSTRACT

This protocol is used to get the abundance of bacteria via flow cytometry from ground, decomposing grass leaf litter.

DOI

dx.doi.org/10.17504/protocols.io.buznnx5e

PROTOCOL CITATION

cweihe 2021. Bacterial abundance from grass litter - Flow cytometry. **protocols.io** https://dx.doi.org/10.17504/protocols.io.buznnx5e

CREATED

May 13, 2021

LAST MODIFIED

Jun 11, 2021

PROTOCOL INTEGER ID

49934

MATERIALS TEXT

1. 50 ml tubes
2. 15 ml tubes
3. ultrasonic bath sonicator
4. Flow cytometer (Novocyte, Agilent)
5. pipet tips 1-10 ul
6. pipet tips 5 ml
7. pipet tips 100-1000 ul
8. amber eppendorf tubes 1.5 ml
9. vacuum manifold, Hoefer FH225
10. 2.7 um glassfiber filter, Whatman 1823-025
11. 0.9% Saline

12. **200x. Sybr Green**
Dilute 10000x Sybr Green with TE pH 8.0 to 200x

13. **10% Pi buffered GTA:**

0.4 g NaH2PO4

1.23 g Na2HPO4

dissolve in **80 mL** distilled water

add **20 mL** 50% (wt/wt) Glutaraldehyde

check SDS for Glutaraldehyde

Dilute the 10% solution with 0.9% saline to 1%.

These solutions should be stored in the dark at **4 °C**

14. **100 mM Tetrasodium Pyrophosphate** (TSP, Na4P2O7.10H2O)

Weigh out **4.46 g** of TSP in 80 ml distilled water

Warm solution slightly and let powder dissolve. Do NOT boil!!

Let cool and measure pH. Adjust pH to **pH 8.0** and fill volume to **100 mL**

1 Add **5000 µl** of **[M] 1 % (v/v)** Pi-buffered GTA solution to **0.1 g** ground grass litter sample in a 50 ml tube.

2 store fixed sample in the dark at **4 °C** for up to 30 days

3 When you are ready to run your samples on the flow cytometer add **550 µl** of 0.1M TSP buffer **pH 8** and vortex.

4 Put samples from step 3 in ultrasonic bath at **4 °C** for 30 min.

Citation: cweihe (06/11/2021). Bacterial abundance from grass litter - Flow cytometry. https://dx.doi.org/10.17504/protocols.io.buznnx5e

5 Prepare vacuum manifold (Hoefer FH225) by placing 15 ml tubes on the bottom of manifold in a holder. Make sure filtration nozzles of the filtration unit lead into the 15 ml tubes.

If you have more than 10 samples to filter keep the remaining samples at **4 °C** in the dark until they can be filtered.

Add 25 mm 2.7 um filter on the filter support grids and then place the chimney weights on top.
Make sure the tube from the pump is connected with the vacuum connector. Turn on vacuum pump and pour samples from step 4 into the chimney weight and open the teflon valves. Wait until all liquid is filtered through.
After the filtration remove chimney weights carefully, remove top part of the manifold and close all the 15 ml tubes.
Take out the 15 ml tube holder from the filtrate collection box. Replace the top on the filtrate collection box and rinse the filtration support grid with DI water. Then rinse with ethanol let vacuum run for a while to dry. In the mean time clean chimney weights with soap and water and dry them. Then clean the chimney weights with ethanol. Re-assemble for more filtrations.

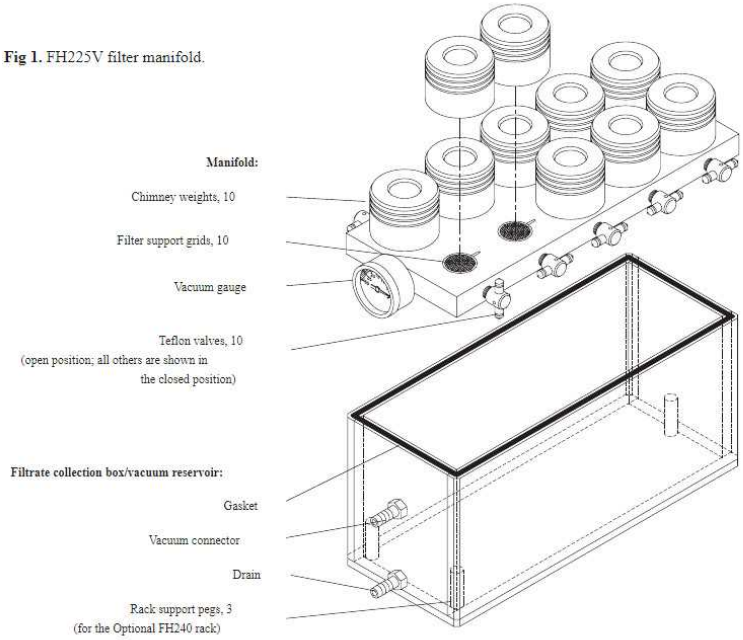

Fig 1. FH225V filter manifold.

6 Take the 15 ml tubes from step 5 vortex sample and transfer **600 µl** into a dark microcentrifuge tube. From there prepare a 1:10 dilution with a total volume of **600 µl** into a new tube with 1% Pi-buffered GTA.

7 Stain the 1:10 diluted sample with 3 ul of 200 x SYBR Green **Thermo Fisher Scientific** nucleic acid stain and vortex. Final concentration of Sybr green should be 1x. Incubate at **Room temperature** for 15 min.

8 On the Novocyte Flow cytometer use green fluorescence channel (FL1) and forward scatter (FSC-H) detectors to reduce the natural autofluorescence found in environmental samples. Vortex each sample before running on the Flow cytometer.
Each sample should be run at 40 ul / min for 1 min. Make sure that the events/sec stay between 100-1500 events/sec to get more reliable counts. Samples might need to be diluted differently if this number gets too high.
Run unstained Pi-buffered GTA as a control for autoflourescence of the buffer. And stained Pi-buffered GTA as a control that the buffer was not contaminated. Run a few unstained samples to account for autoflouresence from environmental conditions.

Citation: cweihe (06/11/2021). Bacterial abundance from grass litter - Flow cytometry. https://dx.doi.org/10.17504/protocols.io.buznnx5e

9 Take absolute counts (evnets/ul) from the gate and subtract the absolute counts within the gate from the background sample. Multiply this by the dilution factor and finally by 1000 to get counts/ml.

Citation: cweihe (06/11/2021). Bacterial abundance from grass litter - Flow cytometry. https://dx.doi.org/10.17504/protocols.io.buznnx5e