Protocol for counting pathogen spores on hemocytometer V.2

Meghan Duffy[1]

[1]University of Michigan - Ann Arbor

2

Apr 12, 2022

1

document .

Duffy Lab, EEB, University of Michigan

kmonell

This is a protocol to quantify the number of pathogen spores you have in a sample using a hemocytometer.

Meghan Duffy 2022. Protocol for counting pathogen spores on hemocytometer. **protocols.io** https://protocols.io/view/protocol-for-counting-pathogen-spores-on-hemocytom-b7harj2e kmonell

document ,

Apr 12, 2022

Apr 12, 2022

60674

This is a protocol to quantify the number of pathogen spores you have in a sample using a hemocytometer.

Protocol for counting pathogen spores on hemocytometer

1. Rinse hemocytometer and glass covering with 90% ethanol and dry off with kimwipe.
2. Place dead infected *Daphnia* in 1.5 ml centrifuge tube, and suck out all excess water with a glass pipette.
3. Fill Pipette with a known volume of DI water. If you are doing spore counts for a single *Daphnia*, 0.1 ml of water is most appropriate. If you are doing spore counts for multiple *Daphnia* at a time, then 0.5 ml is most appropriate. Do not fill to 1 ml or above, as this will lead to water splashing and overflow during homogenization process.

4. With a motorizes pestle, grind up *Daphnia* in DI water for approximately one minute. Throughout process, use pestle to push water collecting on sides of tube into the bottom so that the entire mixture can be properly homogenized.
5. Pipette between 10 and 20 µl of solution into one side of hemocytometer. Pipette enough that the fluid covers the counting chamber, but not so much that you dislodge the cover plate. The exact amount of fluid needed depends on the exact positioning on of cover plate.
6. Wait ~ 1 minute for the spores to settle. If you look through the microscope at 40x magnification and see different sets of cells when you slightly adjust the focus, then you need to wait longer.
7. In the middle of the hemocytometer is a 3 by 3 grid of 9 1mm x 1mm squares, each subdivided into smaller grids. You will focus on the corner squares that are each subdivided into 16 0.25 mm x 0.25 mm squares. (consult diagram of hemocytometer). You will count spores within each of these corner squares, resulting in 4 counts.
8. Calculation for spores per *Daphnia* is then

spore/daphnia=average count*10,000*solution volume(ml)