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Protocol status: In development
We are still developing and optimizing this protocol

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PFA treatment of OP50

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ABSTRACT

Paraformaldehyde (PFA), a polymer of formaldehyde, is an organic solution that permeabilizes bacterial cells making them no longer viable/metabolically active. However, PFA does not cause the lysis of inner cell structure, meaning the bacteria are still edible for the worms. Adapted from Beydoun *et al.* 2021.

MATERIALS

Paraformaldehyde Solution, 16% (15799150) - stored in safety cabinet

PROTOCOL integer ID:
76681

PFA solution

1 In the fume hood, break off the head of the ampoule of 16% paraformaldehyde (PFA)

2 Using a plastic Pasteur pipette, decant the solution into a brown glass bottle. Dispose of Pasteur pipette in Biobin in fume hood.

3 Leave the ampoule on the plastic tray in the fume hood to allow any remaining PFA to evaporate. Dispose of in sharps bin.

4 Store the PFA stock solution double contained at 4°C

PFA treatment

5 Follow steps 1 to 12

Protocol

NAME

Growing overnight culture of OP50 as worm food

CREATED BY

Bonnie Evans

PREVIEW

6 In the fume hood, add 16% PFA stock to the culture to give a 0.5% concentration

Safety information

If a spill occurs within the fume hood, the spill can be considered minor and can be cleaned up using paper towels. Clean any contaminated surface with cold water at least two times. Place the contaminated paper towels into a sealable bag for disposal as hazardous waste.

7 Incubate culture for 2 hours at 37C 200rpm

8 Divide culture into an even number of 50mL Falcons with equal volume

Note

It is important to use sterile technique to prevent the introduction of live bacteria into the culture

9 Centrifuge for 20 minutes at 4000 rcf 4C

10 Pour off supernatant into a waste flask

Safety information

Treat with Virkon when finished

11 Using a stripette, replace with an equal volume of M9 and re-suspend pellet

12 Repeat steps 6 to 8 twice more

13 Vortex Falcons to fully re-suspend pellet and combine solutions in a larger container (should be the same total volume as the original culture).

14 Measure OD600 using M9 as a blank

15 Using an inoculation loop, streak the PFA-treated culture on LB agar and incubate overnight at 37C to check for contamination/live bacteria

16 Store PFA culture at 4C

17 Once PFA killing has been confirmed as successful, aliquot into 50mL Falcons and store at 4C

Safety information

Dispose via hazardous waste