

Protocol for Using Freeze-Dried OP50

Product Specifications:

Features	Details
Strain:	OP50 (<i>Escherichia coli</i>), Uracil auxotroph. E. coli B. Biosafety Level: BSL-1.
Substance:	Powdered (freeze-dried)
Monoculture:	Empty vials were Gamma rayed 25 kiloGray (kGy), Monoculture OP50 present in the vials was not Gamma rayed
Density:	Content: 1e+12 cells, whereof 5e+8 viable
Solubility:	Good - Color: Turbid yellow when dissolved in H2O/S-medium
pH:	6.8-7.5
Shelf Life:	October 28, 2021 for batch # LT20191028
Cultivation methodology:	n.a.
Contents cultivation medium*:	n.a.
Storage Freeze-Dried):	-20°C
Storage Resuspended:	4°C

*If you only need a small amount of freeze-dried OP50, we would like to advise you to **only suspend half of the vial.** The 'dry powder' is the most stable and consistent way to store the OP50.



Component A:
Freeze-Dried OP50



Component B:
Reusable Filtration kit with syringe



Kit assembly
Stack the 3 components
(Yellow, white, green)

Materials included:

- Component A: Freeze Dried OP50
- Component B: Reusable filtration kit*
 - 1x 100 µm filter (yellow)
 - 1x 70 µm filter (white)
 - 1x adapter ring (green)
 - 1x 60 mL Luer Lok syringe

*The number of kits included will depend on the amount of OP50 you ordered.
If you need more, please contact support@nemametrix.com

Materials NOT included but needed:

- 1x 50 mL centrifuge tube
- 40 mL demineralized H₂O
- 210 mL S-medium (if using for liquid cultures)

Preparation:

1. Remove the seal and place in a sterile environment. Allow the vial to warm to room temperature (~20 min).
2. Assemble kit components for filtration. The kit should look like the image above titled "kit assembly". Be sure to securely stack the filters and adapter together. Once secure, insert the syringe into the green adapter. Twist the syringe until it is snug. The purpose of the syringe is to increase filtration speed. **NOTE: Liquid will NOT flow into the syringe.**
3. Place the assembled kit and syringe on a new 50 mL conical tube. Be sure that it is securely on the tube.
4. Remove the cap on the OP50 and aseptically add sterile demineralized water to the 40 mL mark. Place cap back on tube.
5. Mix thoroughly/shake for 30 sec and allow bacteria to rehydrate for at least 5 min at room temp.
6. Mix then slowly pour the OP50 suspension through the top of the filtration device. As it slows, gently pull the syringe so that it continues to flow. Continue until all of the suspension has been filtered. **NOTE: Liquid will NOT flow into the syringe.**

7. You will have particulate material on the filters when you are complete. The filters can be washed in water and EtOH.

We recommend washing the filters immediately after use or material will become trapped in the mesh and unusable.

8. Place cap back on filtered suspension.
9. Your OP50 is now ready to use!

Plating

For 60mm dishes

1. Use 400uL OP50 solution for each 60mm NGM plate and allow to dry in flow cabinet.

250mL Liquid Culture

1. Follow the same procedure above except add to 210 mls of S-medium to the 40 mLs of filtered OP50.

Watch the protocol preparation video [HERE](#) or scan the QR code below!



If you have any questions, please contact support@nemamatrix.com