

Blackwell Lab

Rapamycin Plates Protocol

Monet Bland

For 250 mL NGM (~25 medium plates(6cm)):

- 400 μ L 100% Cholesterol
- 250 μ L 1M CaCl₂
- 250 μ L 1M MgSO₄
- 6.25 mL Phosphate Buffer (pH = 6.0)
- 500 μ L Rapamycin solution
- 250 μ L 100 mg/mL FUDR (if needed)
- 100 μ L 1M IPTG (if needed)

For Rapamycin (LC Laboratories, Cat. No. R5000) Solution:

- 22.85 mg Rapamycin in 500 μ L 100% Dimethyl Sulfoxide (Fisher Scientific, CAT. NO. BP231-1)

Protocol:

(1) Autoclave NGM (250 mL). Refer to protocol at:

http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html

(2) Heat water bath to 55° C.

(3) Once NGM has cooled to 55° C, put NGM into water bath and add necessary reagents (see above) including rapamycin. Adding the rapamycin when the NGM is too hot will result in “frying” the rapamycin.

(4) Mix the NGM by swirling the bottle/flask.

(5) Keep NGM in the water bath while pouring plates.

Important!

- Addition of rapamycin causes a chemical reaction that results in a white precipitate.
- Rapamycin plates are very stable and will work well for at least 4 weeks, when kept at 4°, but it is recommended to use them as soon as possible.
- Don't forget to make DMSO control plates in parallel. Use the above protocol but do not add rapamycin to the 500 μ L of DMSO.

For Lifespans: Put day 1 adults on rapamycin plates and continue treatment until death.

For Stress Assays: Put late L4 worms onto rapamycin plates and treat for three days. Perform the stress assay after the three-day treatment.