

Hygromycin Selection Protocol for Plants

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Hygromycin B from Bioshop stock = 100mg/ml

Working concentration = 20ug/ml

Therefore, 100ul of stock / 500ml AT media

- 1) Put seeds out on Hygromycin 20ug/ml plates
- 2) Keep plates at 4°C wrapped in foil paper for 2-3 days
- 3) Move plates to continuous light for 6-12hrs at RT (better to do it for 12 hrs)
- 4) Put plates in the dark again for 2-3 days but this time at RT
 - Sensitive seedlings – short hypocotyls; bleached cotyledons; little or no germination
 - Resistant seedlings – long hypocotyls; green cotyledons

NB: If no resistant seedlings show up even in positive control population then repeat steps 3 and 4 once again.

- 5) Keep at bench in low light for a day to let seeds recover
- 6) Then transfer plates to growth chamber until ready to transplant into soil.