

## Hygromycin B Solution

### Quick Reference Protocol

Instructions for MIR 5930

SDS and Certificate of Analysis available at [mirusbio.com/5930](http://mirusbio.com/5930)



## SPECIFICATIONS

<b>Storage</b>	Store Hygromycin B solution at 4°C. Protect from moisture.
<b>Product Guarantee</b>	As labeled on the product, when properly stored and handled.
<b>Concentration</b>	50 mg/ml Hygromycin B Free Base, sterile filtered in PBS.
<b>Handling</b>	<b>TOXIC.</b> Handle with care. See SDS for more information.

### ▶ ANTIBIOTIC KILL CURVE PROTOCOL



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Hygromycin B antibiotic ensures effective positive selection of cells expressing the hygromycin resistance (*hph*) gene. In mammalian cells, the recommended working concentration range for hygromycin B is 100 – 500 µg/ml. Different cell types and cell culture conditions may require different concentrations of selection antibiotic. Perform a kill curve to determine the optimal working concentration for your experiment. The following is a general guideline for performing an antibiotic kill curve.

**NOTE:** Performing a kill curve is recommended with each new cell type or selection antibiotic lot, or if changes are made to the cell culture conditions.

- A. Plate cells in 0.5 ml complete growth medium per well in a 24-well tissue culture plate.  
**For adherent cells:** Plate cells at a density of  $0.8-3.0 \times 10^5$  cells/ml.  
**For suspension cells:** Plate cells at a density of  $2.5-5.0 \times 10^5$  cells/ml.
- B. Culture overnight. Most cell types should be  $\geq 80\%$  confluent prior to adding the selection antibiotic.
- C. Add increasing amounts of hygromycin B to duplicate wells of cells plated in complete media. Include a no-antibiotic control. For example, add 0, 25, 50, 100, 150, 200, 250, 300, 400 and 500 µg/ml hygromycin B to duplicate wells of cells plated in complete growth media. Certain cell types and cell culture conditions may require concentrations outside of this range.
- D. Replace media containing selection antibiotic every 2-3 days for up to a week. Examine the culture every day for signs of visual toxicity. Determine the following antibiotic doses:
  - **Low dose** - the antibiotic concentration at which minimal visual toxicity is apparent after 7 days of antibiotic selection
  - **Optimal dose** - the lowest antibiotic concentration at which all cells are dead after 7 days of antibiotic selection
  - **High dose** - the antibiotic concentration at which visual toxicity is evident within the first 2-3 days of antibiotic selection
- E. Proceed with stable cell line generation using the concentrations determined in step D. Cells transfected with a plasmid harboring the hygromycin resistance (*hph*) gene should be grown in complete growth medium for 48-72 hours post-transfection before selection antibiotic is applied. For more information on stable cell line generation, visit [www.mirusbio.com/stable](http://www.mirusbio.com/stable).

## ▶ NOTES

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Rev0 01282022

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