

# Puromycin Dihydrochloride Solution

## Quick Reference Protocol

Instructions for MIR 5940

MSDS and Certificate of Analysis available at [mirusbio.com/5940](http://mirusbio.com/5940)



### SPECIFICATIONS

<b>Storage</b>	Store Puromycin Solution at $-20^{\circ}\text{C}$ . Protect from moisture.
<b>Product Guarantee</b>	As labeled on the product, when properly stored and handled.
<b>Concentration</b>	10 mg/ml Puromycin Dihydrochloride, sterile filtered in DI water

#### ► ANTIBIOTIC KILL CURVE PROTOCOL



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Puromycin antibiotic ensures effective positive selection of cells expressing the puromycin-N-acetyl-transferase (*pac*) gene. In mammalian cells, the recommended working concentration range for puromycin is 0.5 – 10  $\mu\text{g/ml}$ . 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 x 10<sup>5</sup> cells/ml.  
**For suspension cells:** Plate cells at a density of 2.5—5.0 x 10<sup>5</sup> cells/ml.
- B. Culture overnight. Most cell types should be  $\geq 80\%$  confluent prior to adding the selection antibiotic.
- C. Add increasing amounts of puromycin to duplicate wells of cells plated in complete media. Include a no-antibiotic control. For example, add 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0  $\mu\text{g/ml}$  Puromycin 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 puromycin-N-acetyl-transferase (*pac*) 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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