

Puromycin Dihydrochloride Solution

Quick Reference Protocol

Instructions for MIR 5940

MSDS and Certificate of Analysis available at mirusbio.com/5940



SPECIFICATIONS

Storage	Store Puromycin Solution at -20°C . Protect from moisture.
Product Guarantee	As labeled on the product, when properly stored and handled.
Concentration	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 $\mu\text{g}/\text{ml}$. 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 10⁵ cells/ml.
For suspension cells: Plate cells at a density of 2.5—5.0 x 10⁵ 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 $\mu\text{g}/\text{ml}$ 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.

▶ NOTES

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