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 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 x 10^5 cells/ml.
For suspension cells: Plate cells at a density of 2.5—5.0 x 10^5 cells/ml.

B. Culture overnight. Most cell types should be ≥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 µg/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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