

G418 Sulfate Solution

Quick Reference Protocol

Instructions for MIR 5920

MSDS and Certificate of Analysis available at mirusbio.com/5920



SPECIFICATIONS

Storage	Store G418 Sulfate Solution at 4°C. Protect from light.
Product Guarantee	As labeled on the product, when properly stored and handled.
Concentration	50 mg/ml G418 Sulfate, sterile filtered in DI water

▶ ANTIBIOTIC KILL CURVE PROTOCOL



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G418 antibiotic ensures effective positive selection for cells expressing the neomycin resistance (*neo*) gene. In mammalian cells, the recommended working concentration range for G418 is 0.1 – 2.0 mg/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 ≥80% confluent prior to adding the selection antibiotic.
- C. Add increasing amounts of G418 to duplicate wells of cells plated in complete media. Include a no-antibiotic control. For example, add 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1.0, 1.2, 1.5 and 2.0 mg/ml G418 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 neomycin resistance (*neo*) 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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Rev.A 1216