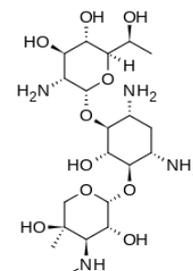


G-418 Sulphate Solution (50mg/ml)

#GTC12.0010
 (for research only)

G-418 (G418 or Geneticin) is an aminoglycoside antibiotic from *Micromonospora rhodorangea*, related to Gentamicin, which acts by inhibiting protein synthesis. It exhibits toxicity towards both prokaryotic and eukaryotic cells, allowing for the selection and maintenance of cells harbouring the Tn5-derived neomycin resistance gene (*neo*) encoding amino-glycoside-3'-phosphotransferase.



Specifications

Quantity:	10ml solution of G-418 disulfate at a concentration of 50mg/ml
Potency:	100% Active (1000µg/mg)
Appearance:	If frozen, it should be whitish. Upon thawing, it becomes a transparent liquid.
Sterility:	tested
Endotoxin:	≤ 10EU/ml
Storage:	-20°C. Once defrosted, store at +2°C-8°C for up to 2 months. If needed, one can freeze the solution again, promptly after using. For this, aseptically prepare aliquots of convenient volume, as repeated freeze/thaw cycles should be avoided.

Selection of transfected mammalian cells:

In order to select for stable transfectants, after transfection with a vector harbouring the *neo* gene, cells are incubated in their regular culture medium supplemented with G-418 at an appropriate concentration (see: working concentration). After 48 hours, cells are passed (either direct or diluted) in fresh medium supplemented with G-418. Cells should be passed to fresh medium every 3-4 days and been evaluated for the formation of foci after one week of selection. Depending on the cell line and transfection and selection efficiency, it may take an additional week or longer for foci to develop. After selection, pool 5-10 positive clones and transfer to a 35mm cell culture plate and maintain on selection medium for another 7 days, before expanding for subsequent assays. G-418 should not be used in combination with other antibiotics/antifungal preparations (such as Pen/Strep), as they are potentially competitive inhibitors of G-418.

Working Concentration:

A typical working concentration of G-418 for most mammalian cells is 400-1000µg/ml. In general, the concentration used for cell line maintenance is ≤50% of the concentration required for selection. Optimal concentration of G-418 required to select or maintain cells depend on the cell line as well as on growth conditions, and should be determined for each case. For this, a kill curve should be made to determine the minimum concentration to kill untransfected, non-resistant cells in their regular growth medium. A comprehensive list of published concentrations of G-418 for many cell lines/culture media can be found at http://cell-lines.toku-e.com/Antibiotics_14.html

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