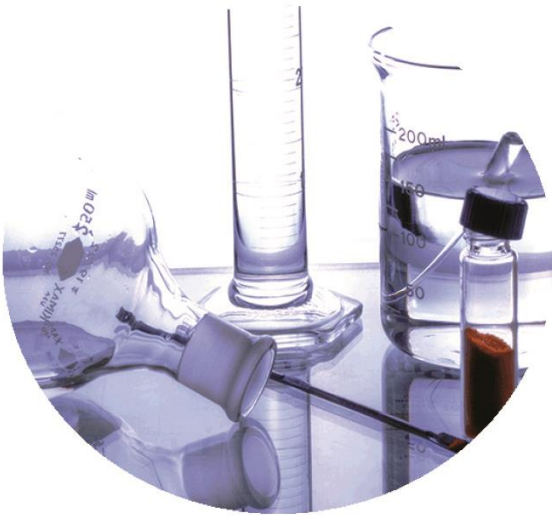




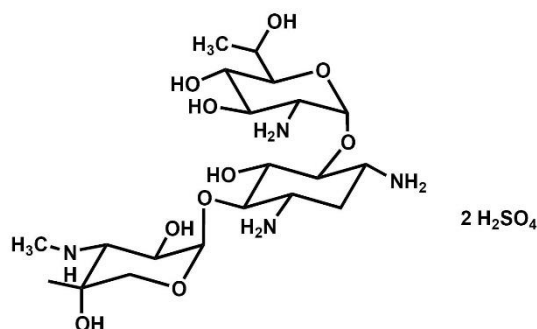
Transfection tools

G418 Sulfate

Protocol

IMPORTANT NOTES – Before you begin

- ✓ The G418 sulfate is an aminoglycoside antibiotic identical to gentamicin B1 produced by *Micromonospora rhodorangea*. It blocks polypeptide synthesis by inhibiting the elongation step in both prokaryotic and eukaryotic cells. It is used to select and maintain eukaryotic cells expressing the neo gene (neomycin).
- ✓ G-418 resistance conferred by the neo gene encoding an aminoglycoside 3'-phosphotransferase, APH 3' II is due to the inactivation of G-418 by covalent modification of its amino or hydroxyl functions. In contrast to gentamicin, G-418 blocks protein synthesis in eukaryotic cells by irreversibly binding to ribosomes and disrupting their proofreading capacity.
- ✓ **G-418 Sulfate:**
 - Chemical Name: (2R,3S,4R,5R,6S)-5-amino-6-[(1R,2S,3S,4R,6S)-4,6-diamino-3-[(2R,3R,4R,5R)-3,5-dihydroxy-5-methyl-4-methylaminooxan-2-yl]oxy-2-hydroxycyclohexyl]oxy-2-(1-hydroxyethyl)oxane-3,4-diol
 - Molecular Formula: C₂₀H₄₀N₄O₁₀ · 2H₂SO₄
 - Molecular weight: 692.71 g/mol
 - CAS Number: 108321-42-2
 - Molecular biology grade and premium pure.



CAUTION: G-418 is a hazardous compound. Avoid contact with eyes, skin and clothes, harmful if swallowed

For additional information and protocols (optimization, scaling, co-transfection...) tips, troubleshooting or other applications



www.ozbiosciences.com

Any questions?



tech@ozbiosciences.com

G418 sulfate | Specifications

Package content	GS21000 G418 sulfate (1g)
Shipping conditions	The G-418 is shipped at room temperature
Storage conditions	<u>Storage (powder)</u> Upon receipt and for long-term storage, keep the powder in a tightly closed and desiccated container at -20°C. G-418 is stable for at least 2 years at -20°C. <u>Storage (solution)</u> G-418 stock solution can be prepared in molecular biology grade or sterile water. G-418 solution can be either stored at -20°C or +4°C. It is stable for at least one year at +4°C and for two years at -20°C.
Shelf life	1 year from the date of purchase when properly stored and handled
Important notice	For research use only. Not for use in diagnostic procedures.

G-418 is used for selection and maintenance of cells expressing the *neo* gene. It is commonly used for producing and maintaining stably-transfected cell lines. The instructions given below represent successfully applied protocols. They can be used as guidelines to quickly achieve very efficient selection. Optimal conditions do vary according to cell types and culture conditions and the final G-418 concentration might have to be adjusted to achieve best results.

Selection in mammalian cells is usually achieved within three to seven days with concentrations ranging from 0.4 to 1 mg/mL. Cells dividing at higher rate are quickly affected whereas cells with a slow cell cycle required longer time.

1. Preparation of G-418 stock solution

1. In a laminar-flow hood, dissolve 1g of G-418 in 10mL of molecular biology grade water.
2. Filter on 22 μ m by vacuum filtration.
3. We suggest to aliquot the sterile solution of G-418, into small tubes and store them at -20°C or +4°C.

2. Conditions of selection & maintenance for mammalian cells

The effective concentration of G-418 for selection and maintenance of transfected mammalian cell lines with the *neo* gene depends on various factors including cell type and cell culture conditions. Selection in mammalian cells is usually achieved within 3 to 7 days with concentrations ranging from 0.4 to 1 mg/mL.

In a preliminary experiment we recommend to determine optimal concentrations of antibiotic required to kill your host cell line by treating the cells with several concentrations ranging from 100 μ g/mL to 1 mg/mL.

After treatment, cell death occurs rapidly allowing the selection of transfected cells with plasmids carrying the *neo* gene in as little as 7 days post-transfection. Suggested working conditions for selection in some mammalian cells are listed below:

Cell line	Species	Type	G-418 (µg/mL)
293	Human	Kidney	400 - 1000
B16	Mouse	Melanoma	400 - 1000
CHO	Hamster	Ovary (epithelial-like)	200 - 400
H441	Lung epithelial	Human	300 - 500
HeLa	Human	Cervix carcinoma	200 - 800
Jurkat	Human	T Cell line	300 - 500
M1	Mouse	Kidney epithelial cells	300 - 500
MDCK	Dog	Acute T cell lymphocyte	300 - 500
NIH-3T3	Mouse	Fibroblasts	300 - 500

Selection Procedure

G-418 sulfate is generally used at a concentration of 400 µg/ml. However, we recommend testing a range of G-418 concentration to achieve optimum selection.

1. Transfect your cells with a neo gene containing plasmid as usual
2. Incubate your cells at least 48 hours post-transfection in regular growth medium (without G-418). It is important to wait at least 48 hours before exposing the transfected cells to selection media. For suspension cells, we recommend to wait 72h post-transfection before starting the selection.
3. Then, prepare a fresh regular growth medium containing G-418 to select stably transfected cells.
4. Divide or split the cells and cultured them in growth G-418 containing medium.
5. Remove and replace G418-containing medium every 3-4 days.
6. Evaluate cells for foci formation after 7 days of selection. Foci may require an additional week or more to develop depending on the cells and transfection/selection efficiency.
7. Transfer and pool 5-10 resistant clones to a larger dish and maintain on selection medium for an additional 7 days.

Note 1: Antibiotics work best when cells are actively dividing. If the cells become too dense, the antibiotic efficiency will decrease. It is better to have cells at 25-35% confluency when starting the treatment.

Maintenance. G-418 is generally used at a concentration of 200 µg/mL for mammalian cell maintenance.

3. Conditions of use for bacteria and plant cells

G-418 sulfate is generally used at a concentration of 5 -20 µg/mL for bacteria and 10 -50 µg/mL for plant cells.

Additional transfection tools products

- **X-Gal substrate** for staining transformed bacteria and LacZ transfected or infected cells, tissues and organisms
- **D-Luciferin K+ and Na+** for *in vitro* and *in vivo* bioluminescent assays

Purchaser Notification

Limited License

The purchase of the G418 sulfate grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). This reagent is intended for in-house research only by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the G418 sulfate. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all G418 sulfate reagents and documentation to OZ Biosciences, or by destroying all D-Luciferin components. Purchasers are advised to contact OZ Biosciences with the notification that a G418 sulfate is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s). This document covers entirely the terms of the G418 sulfate research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations

G418 sulfate and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

EUROPE & ASIA OZ Biosciences SAS

163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
France

Ph: +33 (0) 486 948 516
Fax: +33 (0) 463 740 015

contact@ozbiosciences.com
order@ozbiosciences.com
tech@ozbiosciences.com

USA & CANADA OZ Biosciences INC

7975 Dunbrook Road
Suite B
San Diego CA 92126
USA

Ph: + 1-858-246-7840
Fax: + 1-855-631-0626

contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com
techUSA@ozbiosciences.com



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