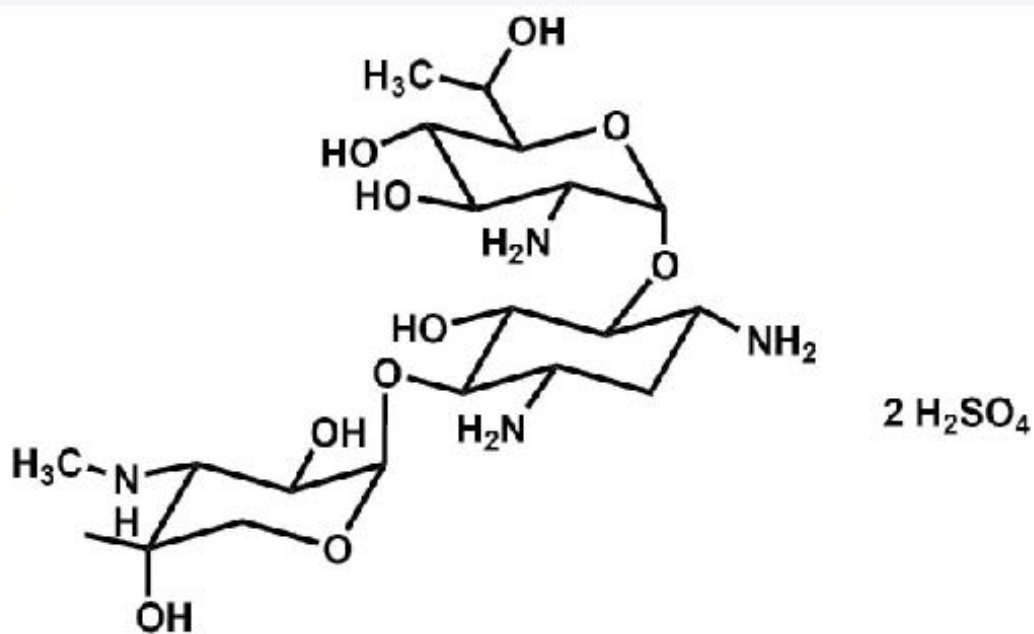


G-418

Product Sheet and Protocol



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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). The quality and purity of the G-418 is essential to achieve good and consistent selection.

List of G-418 product:

Catalog Number	Description	Weight
GS21000	G-418, sulfate	1 g

This product is also available in larger quantities (2g, 5g, 10g etc.). Please contact us for a quotation at order@ozbiosciences.com. For all other supplementary information, do not hesitate to contact our dedicated technical support (tech@ozbiosciences.com).

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1. Description

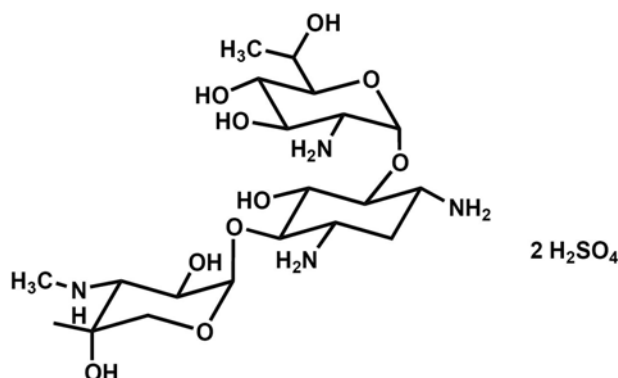
1.1. Introduction and characteristics

Congratulations on your purchase of the **G-418 sulfate antibiotic!**

The **G-418 sulfate** is an aminoglycoside antibiotic related 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.**
- **Molecular Structure:**



1.2. Kit content

G-418, sulfate is provided in a 1.0g/vial. This product is also available in larger quantities (2g, 5g, 10g etc.). Please contact us for a quotation at order@ozbiosciences.com

1.3. Stability and Storage

Storage (powder) 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.

Storage (solution) 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.

Shipping condition The G-418 is shipped at room temperature.

G-418 is a hazardous compound. Avoid contact with eyes, skin and clothes, harmful if swallowed. Please refer to the MSDS for further information.

2. Protocol Examples

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.

2.1. 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 µm 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.

2.2. 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 µg/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.

2.3. 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.

4. Related Products

Description	Reference
Magnetofection Technology	
Mega Magnetic Plate	MF14000
Super Magnetic Plate	MF10000
Magnetic Plate 96-magnets	MF10096
PolyMag 1mL (<i>for all nucleic acids</i>)	PN31000
PolyMag Neo 1mL (<i>for all nucleic acids</i>)	PG61000
CombiMag 1mL (<i>to boost transfection reagent</i>)	CM21000
SilenceMag 1mL (<i>for siRNA application</i>)	SM11000
NeuroMag 1mL (<i>for transfection of neurons</i>)	NM51000
ViroMag 1mL (<i>for all viral applications</i>)	VM41000
ViroMag R/L 1mL (<i>for retrovirus and Lentivirus</i>)	RL41000
AdenoMag 1mL (<i>for adenovirus</i>)	AM71000
SelfMag Amino Kit	SA10000
SelfMag Carboxy Kit	SC20000
FluoMag-P 100µL	FP10100
FluoMag-C 100µL	FC10100
FluoMag-S 100µL	FS10100
FluoMag-V 100µL	FV10100
Protein Delivery Systems	
Ab-DeliverIN 1 mL	AI21000
Pro-DeliverIN 1 mL	PI11000
Lipofection Technology (lipid-based reagents)	
Lullaby siRNA transfection reagent	LL71000
DreamFect Gold Transfection reagent 1mL	DG81000
DreamFect Transfection reagent 1mL	DF41000
EcoTransfect Transfection Reagent 1mL	ET11000
VeroFect Transfection Reagent 1mL	VF61000
FlyFectin Transfection Reagent 1mL	FF51000
CaPO Transfection Kit	CP90000
Plasmids pVectOZ	
pVectOZ-CAT 25µg	PL00010
pVectOZ-GFP 25µg	PL00020
pVectOZ-LacZ 25µg	PL00030
pVectOZ-Luc 25µg	PL00040
pVectOZ-SEAP 25µg	PL00050
Gene & Protein Tools	
Bradford – Protein Assay Kit	BA00100
GeneBlaster selection kit	GB20010
β-Galactosidase (ONPG) assay kits	GO10001
β-Galactosidase (CPRG) assay kits	GC10002
X-Gal Staining Kit	GX10003
Biochemical	
D-Luciferin, Na+ 1g	LN10000
D-Luciferin, K+ 1g	LK10000
X-Gal powder 1g	XG11000

Our dedicated and specialized technical support group will be pleased to answer any of your request and to assist you in your experiments. Do not hesitate to contact us for all complementary information and remember to visit our website in order to stay inform on our last breakthrough technologies and updated on our complete product list. <http://www.ozbiosciences.com>.

Purchaser Notification

Limited License

The purchase of the **G-418** product grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in section 1, Kit Contents). This reagent is intended **for internal research only** by the buyer. Such use is limited to the use 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.

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The **G-418** product 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 handling of the kit components by following proper research laboratory practices.

For more information, or for any comments on the terms and conditions of this License, please contact:

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