G418 Sulfate (Geneticin)

Product Information

Product Name	Cat#	Size
G418 Sulfate (Geneticin)	60220ES03	1 g
	60220ES08	5 g

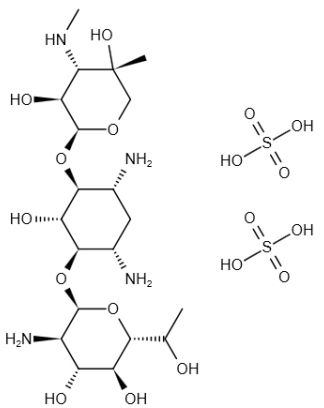
Product Description

G418 Sulfate, also known as Geneticin Sulfate, is an amino-glycoside antibiotic and structurally similar to Gentamycin B1. It interfers with protein synthesis by binding 80s ribosome and blocking elongation steps. G418 Sulfate is toxic to both prokaryotic and eukaryotic cells, including bacteria, yeast, higher plant and mammalian cells, as well as protozoans and worms. The resistance gene (mainly neo), locating in transposon Tn601 (903) or Tn5, is derived from bacteria, but can be expressed in eukaryotic cells. The resistance gene can be introduced into cells through gene recombination techniques to obtain resistance of G418, which could be used to screen and maintain the culture of prokaryotic or eukaryotic cells carrying resistance gene.

In mammalian cells, neo,encodes the expression of amino-glycoside 3' -phosphotransferase (APH (3') II) after integrated into the eukaryotic genome. This enzyme inactivates the antibiotic by covalently modifying the amino or hydroxyl function of G418 and inhibiting the antibiotic-ribosome interaction, endowing the cell with G418 resistance. In screening stable cell line experiments, the killing curves (dose-response curves) should be established to determine the minimum effective concentration for killing non-resistant cells.

In plant cells, resistance can be obtained by transfection of nptII gene resistant plasmid. The nptII gene also encodes aminoglycoside phosphotransferase, an enzyme that inactivates several antibiotics, including G418, kanamycin, and palomycin.

Product Properties

Synonym	G-418 disulfate; Geneticin sulfate; Antibiotic G418; G 418 Sulfate; Antibiotic G-418 sulfate
CAS No.	108321-42-2
Molecular formula	$C_{20}H_{40}N_4O_{10}\cdot 2\ H_2SO_4$
Molecular weight	692.7 g/mol
Appearance	White or light white powder
Purity	≥98%
Potency（anhydrous）	> 700 U/mg
Solubility	≥60 mg/mL in H_2O; insoluble in EtOH; insoluble in DMSO
Structure	

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Shipping and Storage

The product is shipped with ice pack and can be stored at -20℃ for 3 years.

Avoid moisture, which can reduce antibiotic activity.

Cautions

1. This product can not be autoclave.
2. G418 can not be used with other antibiotics/antifungals (e.g. penicillin/streptomycin) as they are competitive inhibitors of G418. Other antibiotics potentially cross-reactive as well.
3. When preparing G418 solution, it is necessary to make conversion based on different potency values of Batch G418, so as to obtain storage solution and working solution requiring activity concentration.
4. When G418 is added into the culture system, the untransfected cells may not be killed, possibly because the concentration is too low or the cell density is too high. In addition, cells that divide quickly are more likely to be killed than cells that multiply slowly.
5. Even with the addition of the killing dose of G418, cells may continue to divide 2-3 times. The effects of G418 usually take two days to become apparent.
6. For your safety and health, please wear lab coats and disposable gloves for operation.
7. For research use only!

Instructions

1. Preparation of G418 storage solution (50 mg/mL, active concentration)

1) Conversion of activity units

Conversion was performed according to this formula: (1000/A0) ×A1=A2

A0: Potency value of G418, which varies from batch to batch. See the batch quality report or label on the bottle.

A1: The concentration of active G418 you desire.

A2: The actual concentration of G418.

For example, if the G418 activity value of the batch is 750 U/mg and the active concentration of G418 is 50 mg/mL, the actual powder concentration to be prepared is 1000/750×50 mg/mL=66.33 mg/mL. If 10 mL of G418 storage solution (active concentration, 50 mg/mL) is prepared, 663.3 mg of powder should be weighed.

2) Sterilization and preservation

Weigh the actual powder obtained by the above conversion and add 10 mL of sterile deionized water to dissolve it completely.

Pre-wet 0.22 μm needle filter with 5 mL of sterile deionized water, and then sterilize G418 solution by filtration. Aliquot the sterilized G418 solution and store at -20℃.

【Note】 ① Do not filter the cloudy solution, as the solution has not completely dissolved, the filtration process will cause drug loss, reduce the activity of the final solution. ② It is not recommended to use culture media, phosphate solutions or organic solvents to prepare the storage solution.

2. Commonly used screening concentration

In general, a high concentration of G418 is required initially for screening transforters and use a low concentration to maintain culture. Growth conditions, cell types, and other environmental factors may affect the effective dosage of G418, so it is recommended to determine the optimal screening concentration through the killing curve (dose-response curve).

Generally, the screening range of mammalian cells is 200-2000 μg/mL, Plant cell: 10-100 μg/mL, Yeast cell: 500-1000 μg/mL.

Cell Line Experimental Data

cell type	Activate the concentration	Application	References
Dictyostelium	a) 10 μg/mL b) 30 μg/mL	a) Culture in medium b) Culture on lyophilized bacteria	Hirth,et. al., *Proc. Natl. Acad. Sci.*, v. 79, 7356-7360 (1982).
Mammal Species	a) 400 -1000 μg/mL b) 200 μg/mL	a) Used for screening b) Used for maintenance	Canaani and Berg, *Proc. Natl. Acad. Sci.*, v. 79, 5166-5170 (1982).

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Plant	a) 25-50 μg/mL b) 10 μg/mL	a) Used for screening b) Used for maintenance	Ursic, et. al., *Biochem. Biophys. Res. Comm.*, v. 101:3, 1031-1037 (1981).
Yeast	a) 500 μg/mL b) 125-200 μg/mL	a) Used for screening b) Used for maintenance	Jimenez and Davies, *Nature*, v. 287, 869-871 (1980).
Bacteria	16 μg/mL	Used for screening	Waitz, et. al., *Antimicrob. Agents Chemother.*, v. 6:5, 579-581 (1974).

3. Establishment of killing curve

【Note】In order to screen for cell lines with stable expression of target proteins, it is necessary to determine the minimum concentration of antibiotics that can kill untransfected host cells. This can be achieved by establishing a killing curve (dose-response curve). At least six concentrations should be selected. G418 is most active when treating mitotic cell, so cells need to be cultured for a period of time before adding G418.

1) Day 1: Untransformed cells are placed on an appropriate culture plate with a cell density of 20-25% at 37℃ and cultured overnight in CO_2;

【Note】For cells requiring higher density to detect viability, the amount of inoculation can be increased.

2) Set the G418 concentration gradient within the appropriate range according to the cell type. Mammalian cells can be set at 0, 50, 100, 200, 400, 800, 1000 μg/mL.

3) Day 2: Remove the old medium and replace it with a freshly prepared medium containing the corresponding concentration of drugs. Make three parallel for each concentration.

4) Then replace with fresh media containing G418 every 3-4 days.

5) Live cell counts are performed at a fixed cycle (e.g., every 2 days) to determine the appropriate concentration. Choose the minimum concentration, which kill the majority of cells within an ideal number of days (usually 7-10 days), as the working concentration for stable transfection cell screening.

4. Screening of stable transfected cells

1) 48 h after transfection, G418 culture medium containing appropriate concentration was used for subculture (direct subculture or diluted subculture).

【Note】In order to obtain good screening results, it is recommended to dilute the cells no more than 25% abundance.

2) Change the screening medium containing drugs every 3-4 days.

3) Measure the cell colony-formation after 7 days of screening. Colony formation may take another week or more, depending on host cell type, transfection, and screening effectiveness.

4) 5-10 resistant clones were selected and transferred to 35 mm cell culture plates, and cultured with drug-containing screening medium for 7 days.

5) Replace with normal medium.

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