

# G418 Disulfate Salt

**Product Code: TC025**

## Product Description:

Molecular Weight: 692.71

Molecular Formula:  $C_{20}H_{40}N_4O_{10} \cdot H_2SO_4$

CAS No: 108321-42-2

Synonym: Geneticin disulfate

Geneticin (G418) is an aminoglycoside antibiotic similar in structure to Gentamicin produced by *Micromonospora rhodorangea*. Geneticin is widely used for selection of eukaryotic cells, stably transfected with neomycin resistance genes (neo) as well as maintenance of the (neor) phenotype of resistance cells. Geneticin is toxic to bacteria, yeast, protozoa, helminths and mammalian cells. It can also be used for elimination of contaminating fibroblasts from mixed cultures.

Geneticin (G418) blocks polypeptide synthesis by inhibiting protein elongation by binding irreversibly to 80S ribosomes both in prokaryotic and eukaryotic cells. This activity can be inactivated by the bacterial aminoglycoside phosphotransferases APH(3')II and APH(3')I encoded by genes on transposons Tn5 and Tn601 (903) respectively. The cells acquire resistance to Geneticin (neor) as a result of transfection of neomycin resistance genes (neo) from transposon Tn5 or Tn601 and enable the cells to grow in media containing Geneticin (G418).

This selection strategy can be used on almost any cell type. The effective concentration of geneticin varies according to the cell type, medium used, growth conditions, cell's metabolic rate and position in the cell cycle. We recommend determining optimal concentrations of antibiotic required to kill your host cell line by treating the cells with several concentrations ranging from 100ug/ml to 1mg/ml. Cells can escape selection if the antibiotic is used at too low concentrations or if the plating density is too high. Ideally cell death of control cells occurs within one week after addition of

antibiotic allowing colonies of resistant cells to form by 10-14 days.

## Directions:

The working concentration of geneticin for selection and maintenance of mammalian cell lines transfected with neo gene depend upon many factors, including the cell type, culture media, metabolic rate of cells and cell cycle phase. The concentration of geneticin to be added to the culture medium has to be determined experimentally.

It is recommended to obtain a full dose curve when-

- a. New lot of geneticin is used
- b. Geneticin is used in new cell culture system

The effective concentrations have been reported from 100µg/ml to 5mg/ml or greater. However, the recommended working concentrations for selection in a few mammalian cell lines are listed below.

Cell line	Species	Geneticin (418) µg/ml
HeLa	Human	150-800
293	Human	500
CHO	Hamster	250-400
BHK	Hamster	500
P3X63Ag.653	Mouse	400
B16	Mouse	400-1000
L929	Mouse	400
L691	Mouse	480
L cells	Mouse	150-500
3T3	Mouse	300-1000
RK13	Rabbit	400
Vero	Simian	150-300
COS	Simian	400-100
RAT1	Rat	400-700
RAT2	Rat	200
A5P/B10	Rat	100

The cells are transfected with plasmid containing neo gene and are incubated in a regular growth medium containing Geneticin to select for stable transfectants. Note that the cells can escape the selection if the antibiotic is used at a too low concentration.

1. Transfect the cells with a plasmid containing neo gene.
2. 48 hours after transfection add the cells in a medium containing Geneticin at appropriate concentration.
3. Replace the antibiotic containing medium every 3-4 days.
4. After 7 days of selection evaluate the cells for the formation of foci. Foci formation may require an additional week or more depending upon the host cell line and transfection efficiency.
5. Transfer and pool 5-10 resistant clones to 35mm cell culture plate and maintain on selection medium for additional 7 days.

### **Quality Control:**

#### **Appearance**

White to off white powder

#### **Solubility**

Clear to slight hazy, colourless to faint yellow solution at 50g/100ml in water.

#### **pH of 20% solution in water**

4.60 -6.00

#### **Water Content**

NMT 12.0 %

#### **Potency**

NLT 650µg/mg

#### **Biological Potency Test (ED<sub>50</sub> Sensitivity Assay)**

NMT 400µg/ml

#### **Biological Potency Test (ED<sub>50</sub> Resistance Assay)**

NLT 2500µg/ml

#### **Antibiotic Sensitivity**

Complies

#### **Cell Culture Test**

Passes

### **Storage and Shelf Life:**

Store at 2-8°C away from bright light.

For long term storage and sterile applications, geneticin solutions should be sterilized by filtering through a membrane with porosity of 0.22microns or less. Solutions remain stable for about 12 months at 2-8°C. For long term storage, store at -20°C.

Shelf life is 36 months.

Use before expiry date given on the product label.

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