

## Selection of pHA-MEX and pMEX-HA vectors using G418 sulfate

---

### PRODUCT DATA

P03401	pHA-MEX	Mammalian expression vector
P03402	pMEX-HA	Mammalian expression vector

### INTRODUCTION

The mammalian expression vectors pHA-MEX and pMEX-HA can be used for transient expression in mammalian cells or for generating stably transfected cell lines using selection with G418 sulfate. The neomycin phosphotransferase (neo) cassette in the backbone of pHA-MEX and pMEX-HA confers resistance to G418 sulfate at concentrations between 100-1000 µg/ml.

### BACKGROUND

G418 is used for the selection and maintenance of eukaryotic cells expressing the *neo* gene (1). G418 is an aminoglycoside antibiotic similar in structure to gentamycin B1, produced by *Micromonospora rhodorangea*. Unlike gentamycin, G418 blocks polypeptide synthesis in eukaryotic cells by binding irreversibly to 80S ribosomes and therefore disrupting their proofreading capability (2).

### RESISTANCE TO G418

Resistance to G418 is conferred by the *neo* gene from transposon Tn5 encoding an aminoglycoside 3'-phosphotransferase, APH 3' II3. This protein inactivates G418 by covalently modifying its amino or hydroxyl functions therefore inhibiting the antibiotic-ribosome interaction.

### CONDITIONS OF SELECTION

#### Mammalian cells

The working concentration of G418 Sulfate for selection and maintenance of mammalian cell lines transfected with the *neo* gene varies with a multitude of factors including cell type. In a starting 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	Tissue	Culture medium	G418 (µg/ml)
HeLa	Human	Uterus	DMEM	200-800
293	Human	Kidney	DMEM	400-1000
B16	Mouse	Melanoma	RPMI	400-1000
CHO	Hamster	Ovary	Ham's	200-400

## METHOD

### (Selection procedure for mammalian cells)

G418 sulfate is normally used at a concentration of 400 µg/ml. After transfection with a plasmid containing the *neo* gene, cells are incubated in their regular growth medium containing G418 to select for stable transfectants.

1- 48 hours post-transfection, pass cells (direct or diluted) in fresh medium containing G418 at the appropriate concentration.

*Note: Antibiotics work best when cells are actively dividing. If the cells become too dense, the antibiotic efficiency will decrease. It is best to split cells such that they are not more than 25% confluent.*

2- Remove and replace antibiotic containing medium every 3-4 days.

3- Evaluate cells for the formation of foci after 7 days of selection. Foci may require an additional week or more to develop depending on the host cell line and transfection/selection efficiency.

4- Transfer and pool 5-10 resistant clones to a 35mm cell culture plate and maintain on selection medium for an additional 7 days. This pooled culture will be expanded for subsequent cytotoxicity assays.

## STORAGE AND STABILITY

G418 sulfate should be stored at 4°C or -20°C. G418 is stable for at least one year at 4°C. For optimal stability store at -20°C.

## SPECIAL HANDLING

G418 is a hazardous compound. Avoid contact with eyes, skin and clothes, harmful if swallowed.

## REFERENCES

1. Davies J & Jimenez A. 1980. A new selective agent for eukaryotic cloning vectors. *Am J Trop Med Hyg* 29(5 Suppl):1089-92
2. Bar-Nun S *et al.* 1983. G-418, an elongation inhibitor of 80 S ribosomes. *Biochim Biophys Acta.* 741(1):123-7.
3. Beck E *et al.* 1982. Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn5. *Gene* 19(3):327-36