

Hygromycin B

Selective antibiotic for the *hph* gene

Catalog # ant-hm-1, ant-hm-5

For research use only

Version # 13J25-MM

PRODUCT INFORMATION

Content:

Hygromycin B is supplied as either 1 ml tubes or a 50 ml bottle of a 100 mg/ml solution in HEPES buffer, pH 7.0. Yellow solution, filtered to sterility for customer convenience, and cell culture tested.

- ant-hm-1: 10 x 1 ml at 100 mg/ml (1 g)

- ant-hm-5: 1 x 50 ml at 100 mg/ml (5 g)

Storage and stability:

- Hygromycin B is shipped at room temperature. Upon receipt, it should be stored at 4°C or at -20°C.

- Hygromycin B is stable for three months at room temperature, two years at 4°C, and two years at -20°C. Avoid repeated freeze-thaw cycles.

- Hygromycin B is sensitive to high concentrations of acid but a short-term exposure to dilute acids can be tolerated.

Quality control

Purity controlled by HPLC: >85%

Activity controlled by bioassays on bacteria and mammalian cell lines.

SPECIAL HANDLING

Hygromycin B is a hazardous compound. Avoid contact with skin and eyes, harmful if swallowed.

BACKGROUND

Hygromycin B is an aminoglycoside antibiotic produced by *Streptomyces hygroscopicus*. It kills bacteria, fungi and higher eukaryotic cells by inhibiting protein synthesis. It has been reported to interfere with translocation¹ and to cause mistranslation at the 70S ribosome^{2,3}.

CHEMICAL PROPERTIES

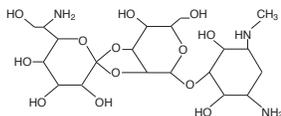
CAS n°: 31282-04-9

Formula: C₂₀H₃₇N₃O₁₃, HCl

Molecular weight: 527.5

pKa values: 7.1 and 8.8 in water.

Structure: The aminocyclitol, N-methyl-2-deoxystreptamine, is linked by a β-glycosidic bond to the talose sugar. This latter moiety is bound by orthoester formation between the hydroxyl group and destomic acid. Hygromycin B is weakly basic.



RESISTANCE TO HYGROMYCIN

Hygromycin B is used as a selective agent in molecular genetics experiments of a wide variety of eukaryotic and prokaryotic species⁴⁻⁹. The *hph* gene confers hygromycin-resistance to cells expressing it and many vectors carrying the *hph* gene are available within the scientific community.

References:

- 1- Cabanas, M. et al. (1978) Eur. J. Biochem. 87, 21-27
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- 3- Singh, A. et al. (1979) Nature 277, 146-148
- 4- Grütz, L. & Davies, J. (1983) Gene 25, 179-188
- 5- Cullen, D. et al. (1987) Gene 57, 21-26
- 6- Sugden, B. et al. (1985) Mol. Cell. Biol. 4, 2929-2931
- 7- Santerre, R.F. et al. Gene 30, 147-156
- 8- Hemmi, S., et al. (1992) Proc. Natl. Acad. Sci. USA 89, 2737-2741
- 9- Mac Gregor, G.R. et al (1987) Somatic Cell. Mol. Genet. 13, 253-265

TECHNICAL SUPPORT

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CONDITIONS OF SELECTION

Most cells growing aerobically are killed by Hygromycin B in the concentration range of 50 to 500 µg/ml. However, the sensitivity of cells is pH dependent, i.e. the higher the pH of culture medium, the greater the sensitivity. Thus, the concentration of Hygromycin B required for complete growth inhibition of given cells can be reduced by increasing the pH of the medium. In addition, using low salt media whenever possible decreases the amount of Hygromycin B needed.

- *Escherichia coli*

Hygromycin-resistant transformants are selected in Low Salt LB agar medium (yeast extract 5g/l, tryptone 10 g/l, NaCl 5g/l, agar 15 g/l, pH 8) supplemented with 50-100 µg/ml of Hygromycin B. Plates containing Hygromycin B are stable for 1 month when stored at 4°C. For optimum results the use of InvivoGen's FastMedia™ Hygro is recommended.

- Mammalian cells

The working concentrations of Hygromycin B for mammalian cell lines vary from 50-200 µg/ml, in a few cases up to 500 µg/ml. In a starting experiment we recommend to determine the optimal concentration of Hygromycin B required to kill your host cell line. The killing and the detachment of dead cells from the plate, specially at high cell density, can require a longer time compared to G418. Foci of hygromycin-resistant stable transfectants are usually individualized after 10 days to 3 weeks incubation, depending on the cell line. Suggested concentrations of Hygromycin B for selection in some examples of mammalian cells are listed below.

Cell line	Species	Tissue	Culture medium	Hygromycin µg/ml
HeLa	Human	Uterus	DMEM	200
293	Human	Kidney	DMEM	100
B16	Mouse	Melanoma	RPMI	50-100
C6	Rat	Glioma	DMEM	100-200
PC1.0	Hamster	Adenocarcinoma	RPMI	100-200

METHOD (Selection procedure for mammalian cells)

HygromycinB is normally used at a concentration of 200 µg/ml, a 500-fold dilution from the stock solution. After transformation with a plasmid containing the *hph* gene, cells are incubated in their regular growth medium containing Hygromycin B to select for stable transfectants.

1- Forty-eight hours post-transfection, pass cells (direct or diluted) in fresh medium containing HygromycinB 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.



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