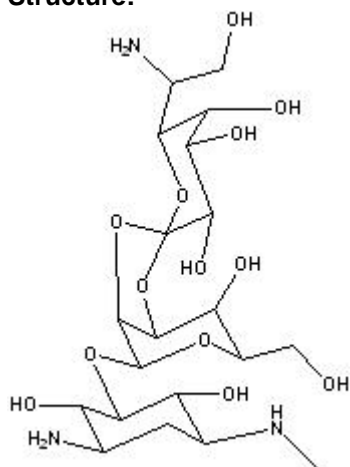


TECHNICAL INFORMATION

Catalog Number: 157513, 194170

Hygromycin B

Structure:



Molecular Formula: C₂₀H₃₇N₃O₁₃

Molecular Weight: 527.5

CAS #: 31282-04-9

Synonyms: O-6-Amino-6-deoxy-L-glycero-D-galacto-heptopyranosylidene- (1->2-3)-O-b-D- talopyranosyl- (1->5)- 2-deoxy-N3-methyl-D-streptamine; Hygromix; E.C. 250-545-5

Physical Appearance: Reddish brown solution

Purity: 96.34%

Formulation (for catalog number 157513): Hygromycin B is provided as a caramel-colored solution in water. Not prepared under sterile conditions. If a sterile solution is desired, working solutions of Hygromycin B in aqueous media can be passed through a 0.2 micron filter before use. Do not autoclave solutions of Hygromycin B.

Bioassay: The activity of Hygromycin B is measured in a zonal inhibition assay using *Bacillus subtilis*. The activity is measured relative to a reference standard.

Solubility: Freely soluble in water, methanol, buffer solution and ethanol. Practically insoluble in less polar solvents.²⁴ Solutions should be sterilized by means of a 0.2 micron filter. Do not autoclave solutions. Hygromycin B is sensitive to high concentrations of acid but short-term exposure to dilute acids can be tolerated.

Working Concentration: When using this product for mammalian cell selection the working concentration is generally between 50 mg/ml and 1 mg/ml, but at times may go as high as 1 mg/ml. The appropriate concentration for use must be determined empirically for each cell type.

The sensitivity of cells to hygromycin B is pH dependent, i.e. the higher the pH, the greater the sensitivity. Suspension cells may be more resistant and therefore may require a higher dosage for selection.

Storage: As provided, Hygromycin B should be stored in the refrigerator (+4°C). Aqueous stock solutions should be kept at concentrations of ≥ 50 mg/ml and can be stored at either +4°C or -20°C.

Stability: Working solutions (< 2 mg/ml) are stable for approximately 1 month at +4°C. Hygromycin B is sensitive to high concentrations of acid but short-term exposure to dilute acids can be tolerated.

Biological Significance:

Hygromycin B, an aminoglycoside antibiotic, inhibits the growth of prokaryotic and eukaryotic microorganisms and mammalian cells. Specifically, it inhibits protein synthesis by interfering with translocation of the 70S ribosome and inducing misreading of the mRNA template.¹⁻³ Hygromycin B has been used to select mutants in a wide variety of cells including bacteria,^{4,5} protozoans,⁶ yeast,⁷ fungi,⁸⁻¹² plants,^{1,13-15} and mammalian cells.¹⁶⁻¹⁸

Resistance to Hygromycin B is conferred by a gene coding for a phosphotransferase that phosphorylates Hygromycin B, thereby inactivating it.^{4,19} Hygromycin B is known to selectively penetrate cells that have been rendered permeable by virus infections.²⁰ This combined with its potency in inhibiting translation, makes it an effective antiviral agent.²¹⁻²³

Hygromycin B is an effective agent for the selection and maintenance of bacterial and eukaryotic cells stably transfected with vectors containing the *E. coli* hygromycin-B resistance gene (hyg or hph). Resistance is conferred by the *E. coli* hygromycin resistance gene by phosphorylation of hygromycin-B-phosphotransferase promoting the activation of the drug.

Hygromycin B can be used as a dual-selection antibiotic when used in conjunction with G418 or Zeocin™ for cells transfected with two different vectors:

Compatible Dual-Selection Antibiotics and Corresponding Resistance Genes

Hygromycin B	Inhibits protein synthesis by disrupting translocation and promoting mistranslation at the 70S ribosome.	hyg or hph
G418 (Geneticin™)	Interferes with the function of the 80S ribosome and blocks protein synthesis.	Tn5 or Tn601
Zeocin™	Causes cell death by intercalating into and cleaving DNA.	<i>Sh ble</i>

Availability:

Catalog Number	Description	Size
157513	Hygromycin B, Aqueous Solution, approximately 450,000 units/ml	250 KU 1 MU 5 MU
194170	Hygromycin B, white powder	50 mg 100 mg 250 mg 1 g

For researchers interested in understanding how the concentration in mg/ml can be calculated from the activity in U/ml we provide the following example as a guideline.

For the 1,000,000 Unit size:

1,000,000 Units at 464,600 U/ml

Volume = 2.16 ml

2.16 ml x 1150 mg/ml = 2484 mg total weight (Hygromycin B + H₂O)

2484 mg total weight x 37.0% (% solids) = 919.08 mg Hygromycin B

919.08 mg Hygromycin / 2.16 ml = **425.5 mg Hygromycin B / ml**

Typical "Kill Curve" Procedure:

To express a recombinant protein of interest in mammalian cells, a mammalian expression vector containing the hyg or hph gene must be introduced into the mammalian host cell line. The hyg or hph marker gene allows for selection of stable transfectants using hygromycin B resistance. As natural resistance to hygromycin B varies greatly among cell lines, MP recommends testing the sensitivity of the mammalian host cell line to hygromycin by generating a "dose curve."

To generate a stable cell line expressing your protein of interest, you need to determine the minimum concentration of antibiotic required to kill your untransfected host cell line. Test a range of concentrations (at least 5) to ensure that you determine the minimum concentration necessary for your cell line.

– Dilute cells in culture medium to 2.0×10^4 cells/ml. Add 4.0 ml of the cell dilution to each well of ten 6-well plates. For cells that require higher densities for viability, proportionally decrease the cell dilution. Allow cells to adhere overnight.

– Prepare a 5.0 mg/ml solution of hygromycin B in distilled deionized water if the antibiotic is in powder form. If the antibiotic is in liquid form, make a 1:200 dilution in distilled deionized water. Make test dilutions of hygromycin B using the following dilution chart:

Final Concentration	Culture Medium	Hygromycin B Solution (5.0 mg/ml)
0.05 mg/ml	29.7 ml	0.30 ml
0.10 mg/ml	29.4 ml	0.60 ml
0.25 mg/ml	28.5 ml	1.50 ml
0.50 mg/ml	27.0 ml	3.00 ml
1.00 mg/ml	24.0 ml	6.0 ml

3. Substitute culture medium with medium containing the varying concentrations of hygromycin B.

4. Count the number of viable cells at regular intervals to determine the appropriate concentration of antibiotic that prevents growth of untransfected cells. Select the concentration that kills the majority of the cells in the desired number of days, generally 7-10 days.

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