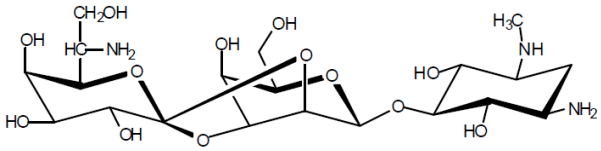
Product Information Sheet

Hygromycin B solution from Streptomyces Hygroscopicus

H0654, H3274, H5527, H7772 and H9773

Product Description



H7772, H3274, H9773: Lyophilized powder

H0654: Aqueous solution 0.2 micron filtered

H5527: Aqueous solution, gamma-irradiated

Melting point: Reported as 160-180 °C (with decomposition).[1]

pK_a values are 7.1 and 8.8.[1]

Hygromycin B is an antibiotic substance isolated from Streptomyces hygroscopicus. Its mode of action is the inhibition of protein synthesis, by inducing the misreading of the m-RNA template in the prokaryote, (E. coli, at 100 µg/mL), lower eukaryotes (For example, yeast, at 200 µg/mL) and higher eukaryotes (For example, mammalian cells in culture). It selectively penetrates cells that have been rendered permeable by virus infection and combined with its potency to inhibit translation, it is an effective antiviral agent.[2,3]

This antibiotic has been used to select drug-resistant stable transfectants after transfer of the hygromycin phosphotransferase gene in a variety of cell culture applications.[4-7] Specific references include transformation of Aspergillus species,[8,9] Agaricus bisporus,[10]Agrobacterium,[11] Histoplasma capsulatum,[12] and Penicillium urticae.[13] For use as a selective agent, the suggested concentration range is 100-800 µg/mL; more specifically, for prokaryotes, 100 µg/mL; lower eukaryotes, 200 µg/mL; higher eukaryotes, 150-400 µg/mL.[14]

Usage of Hygromycin B is generally reported in terms of a mass/volume concentration, but sometimes in terms of units. A former supplier tested the antibiotic in terms of a microbiological assay which included

a) plating a lawn of Bacillus subtilis on agar plates,
b) using a reference preparation of hygromycin to establish a standard curve relating to its "zone of killing" (the area of the bacterial lawn killed)
c) testing a sample of hygromycin to determine its zone of killing.

The units of activity for the first reference preparation of hygromycin were completely arbitrary, and subsequent reference preparations were prepared against that first standard.[2]

Sigma does not perform a biological assay on H7772, H0654, or H5527. Instead, purity is reported by liquid chromatography using pulsed amperometric detection (HPAE). Sigma offers cell-culture tested H3274 and plant cell culture-tested H9773, both of which are tested for bioactivity and ability to select transformed cells.

For protocols that describe usage of Hygromycin B in units, a suggested equivalence is 1 million units is approx. equal to 900 mg H7772, but each lot should be tittered for its application.[2,14]

Preparation Instructions

Hygromycin B is soluble in water, at concentrations < 50 mg/mL, and stable in aqueous solution for at least two years at 2-8 °C.[2] Hygromycin B is also soluble in methanol or ethanol.[1] Aqueous solutions have been found to remain stable for at least a month at 37 °C and 60°C. Solutions should be sterilized by filtration, not autoclaving.[14] Hygromycin B is dissolved in water at 50 mg/mL, then sterile-filtered to produce H0654, or gamma-irradiated to produce H5527.

Although not verified by Sigma, customers have been reported that frozen solution have lost complete activity.[11] Since solutions are extremely stable refrigerated, there should be no need to freeze them.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Stability studies at 37 °C and 60 °C showed no appreciable loss in activity, even for the solution, in one month.[2,10]

References

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