

## Product Information

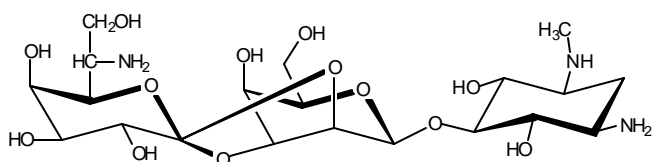
### HYGROMYCIN B

from *Streptomyces hygrosopicus*  
Store at 2-8° C

Product Numbers **H0654, H3274, H5527, H7772 and H9773**

CAS #: 31282-04-09

#### Product Description



H7772, H3274, H9773: Lyophilized powder  
H0654: Aqueous solution 0.2 micron filtered  
H5527: Aqueous solution, gamma-irradiated  
Molecular formula:  $C_{20}H_{37}N_3O_{13}$   
Formula weight: 527.54

Melting point: Reported as 160-180°C (with decomposition).<sup>1</sup>

pK<sub>a</sub> values are 7.1 and 8.8.<sup>1</sup>

Hygromycin B is an antibiotic substance isolated from *Streptomyces hygrosopicus*. 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 (e.g., yeast, at 200 µg/ml) and higher eukaryotes (e.g., 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.<sup>2,3</sup>

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.<sup>4-7</sup> Specific references include transformation of *Aspergillus* species,<sup>8,9</sup> *Agaricus bisporus*,<sup>10</sup> *Agrobacterium*,<sup>11</sup> *Histoplasma capsulatum*,<sup>12</sup> and *Penicillium urticae*.<sup>13</sup> 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.<sup>14</sup>

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

- plating a lawn of *Bacillus subtilis* on agar plates,
- using a reference preparation of hygromycin to establish a standard curve relating to its "zone of killing" (the area of the bacterial lawn killed)
- 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.<sup>2</sup>

Sigma does not perform a biological assay on H7772, H0654, or H5527. Instead, purity is reported by liquid chromatography using pulsed amperometric detection (HPAE). Specifications for H7772 require a minimum purity 60%, although most lots have been somewhat higher in purity. Sigma now produces Hygromycin B at a purity comparable to or greater than that of the products once offered from outside supplier (which were only reported in terms of units/ml or units/mg).<sup>10</sup> 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 titered for its application.<sup>2,14</sup>

#### Preparation Instructions

Hygromycin B is soluble in water, at concentrations >50 mg/mL, and stable in aqueous solution for at least five years at 2-8°C.<sup>2</sup> Hygromycin B is also soluble in methanol or ethanol.<sup>1</sup> 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.<sup>14</sup> 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.<sup>11</sup> Since solutions are extremely stable refrigerated, there should be no need to freeze them.

### Storage/Stability

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

### References

1. *Merck Index*, 12th ed., 4900 (1996).
2. Supplier information.
3. Gonzalez, A. et al., *Biochim. Biophys. Acta*, 521, 459 (1978).
4. Rastinejad, F. et al., *Cell*, 75, 1107-1117 (1993).
5. Gritz, L. and Davies, J., *Gene*, 25, 179 (1983).
6. Malpartida, F. et al., *Biochem. Biophys. Res. Commun.*, 177, 6 (1983).
7. Giordano, T.J. and McAllister, W.T., *Gene*, 88, 285-288 (1990).
8. Wendt, S. et al., *Curr. Genet.*, 17, 21-24 (1990).
9. Cullen, D. et al., *Gene*, 57, 21-26 (1987).
10. Van de Rhee, M.D. et al., *Mol. Gen. Genet.*, 250, 252-258 (1996).
11. Perl, A. et al., *Nat. Biotechnol.*, 14, 624-628 (1996).
12. Woods, J.P. et al, *Infect. Immun.*, 66, 1697-1707 (1998).
13. Kiuchi, N. et al., *Agric. Biol. Chem.*, 55, 3053-3057 (1991).
14. Sigma production and files.
15. Reports from Sigma customers.

ckv 01/07/98

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.