



Hygromycin B, EvoPure (Low Endotoxin)

PRODUCT DATA SHEET

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Product Name:	Hygromycin B, EvoPure (Low Endotoxin)
Product Number:	H024
CAS Number:	31282-04-9
Molecular Formula:	$C_{20}H_{37}N_3O_{13}$
Molecular Weight:	527.52
Form:	Powder
Appearance:	White or off-white powder
Solubility:	Freely soluble
Water Content (Karl Fischer):	Not more than 15.0%
Melting Point:	160-180°C
Description:	<p>Hygromycin B, EvoPure® (Low Endotoxin) has a strict endotoxin content of ≤ 10 EU/mg as characterized by spectral data. The presence of endotoxin in Hygromycin B can decrease transfection efficiency and can even be toxic to resistant eukaryotic cells. This product may be suitable for use as an ancillary material for upstream pharmaceutical manufacturing.</p> <p>Hygromycin B is a unique aminoglycoside antibiotic derived from <i>Streptomyces hygroscopicus</i> routinely used as a selective agent in cell culture or microbiology applications to isolate Hygromycin B resistant cells after transfection or transformation, respectively.</p> <p>This product is considered a dangerous good. Quantities above 1 g may be subject to additional shipping fees. Please contact us for questions.</p>

Mechanism of Action: Hygromycin B inhibits protein synthesis by strengthening the interaction of tRNA binding in the ribosomal A-site. Hygromycin B also prevents mRNA and tRNA translocation by an unknown mechanism. These are unique mechanisms for an aminoglycoside antibiotic and they differ from the mode of action of neomycin, gentamicin, and G418.

Mechanism of Resistance

Hygromycin B resistance is conferred by the *hph* gene isolated from *Streptomyces hygroscopicus*, a 1467 bp fragment which encodes hygromycin B phosphotransferase (HPh). Cell lines successfully transfected with the *hph* gene produce hygromycin B phosphotransferase and convert hygromycin B to 7"-O-phosphoryl-hygromycin B by phosphorylating the 4-hydroxyl group on the cyclitol ring of hygromycin B. 7"-O-phosphoryl-hygromycin B lacks antibiotic activity and does not interact with prokaryotic or eukaryotic ribosomes.

Spectrum: Hygromycin B is effective against eukaryotic and prokaryotic cells.

Microbiology Applications Hygromycin B can be used as a selection agent to isolate Hygromycin B resistant bacteria and fungi. The following Hygromycin B selection concentrations should serve as a guide only and may vary depending on experimental conditions and cells used:

- o Bacteria (*E. coli*) - 50 µg/mL - 100 µg/mL
- o Fungi - 100 µg/mL - 300 µg/mL
- o Yeasts - 50 µg/mL - 200 µg/mL

Plant Biology Applications

Hygromycin B is routinely used as a selection agent for *Arabidopsis* plants that have been transformed with a hygromycin B resistance gene. A rapid method to screen for hygromycin B resistant *Arabidopsis* in less than four days has been developed. After *Arabidopsis* seeds have been transformed with a resistance plasmid (pBIG-HYG), they are plated on MS medium with hygromycin B and subjected to a two day stratification at 4°C in the dark. Seeds are then exposed to light for 4-6 hours to stimulate germination and then placed in the dark for another two days. Transformed seeds are selected and identified after a 24 hour period in the light. Resistant transformants are characterized by long hypocotyls. (Harrison et al, 2006).

References: Dai S et al (2001) Comparative analysis of transgenic rice plants obtained by *Agrobacterium*-mediated transformation and particle bombardment. *Mol. Breeding*. 7: 25–33

Harrison S et al (2006) A rapid and robust method of identifying transformed *Arabidopsis thaliana* seedlings following floral dip transformation. *Plant Methods* 2(19):1-7 PMID 17087829

González A, Jiménez A, Vázquez D, Davies JE, Schindler D. (1978) Studies on the mode of action of hygromycin B, an inhibitor of translocation in eukaryotes. *Biochim Biophys Acta*. 521(2):459-469 PMID 367435