

Hygromycin B
Selection antibiotic; cell culture tested
Sterile solution 100mg/ml in HEPES buffer
Ref. EU3250

Hygromycin B is used as a selective agent in molecular genetics experiments. 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 translocation and to cause mistranslation. The *hph* gene confers hygromycin-resistance to cells expressing it. Many vectors carrying the *hph* are readily available within the scientific community.

Contents:

Ultrapure Hygromycin B. Supplied as a sterile filtered solution at 100mg/ml solution in HEPES buffer.

Lot N°:

CAS N°: [31282-04-9]
Formula: $C_{20}H_{37}N_3O_{13} \cdot HCl$
MW: 564
Appearance: Clear light brown solution
Purity: >90 % Hygromycin B (HPLC)
93.8 % (lot analysis)
pH: 7.8
Endotoxin: 0.082 EU/mg

Microbiological and biological assays:

Antibiogram on bacterial reference strain: 80%-120%

MIC* on hygromycin-sensitive bacteria: <16 µg/ml

MIC* on hygromycin-resistant bacteria: >256 µg/ml

Selective conc. on hygromycin-sensitive mammalian cell lines: <400 µg/ml

Selective conc. on hygromycin-resistant mammalian cell lines: >2000 µg/ml

Long-term cytotoxicity induced by trace contaminants: undetectable after 4 passages

* MIC: minimum inhibitory concentration

Stability/Storage:

Store at -20°C.

Hygromycin B solution is stable for a least 1 year at +4°C and least 1 month at 37°C.

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

Protect from light.

Toxicity:**Toxic.**

Avoid contact with skin and eyes. Avoid inhalation of vapors or mist.

Danger UN2810 6.1 I

H301 Toxique en cas d'ingestion. **H311** Toxique par contact cutané. **H318** Provoque des lésions oculaires graves. **H330** Mortel par inhalation. **H334** Peut provoquer des symptômes allergiques ou d'asthme ou des difficultés respiratoires par inhalation. **P260** Ne pas respirer les poussières/ fumées/gaz/brouillard/vapeurs/ aérosols. **P280** Porter des gants de protection/un équipement de protection des yeux/ un équipement de protection du visage. **P284** Porter un équipement de protection respiratoire. **P301+P310** EN CAS D'INGESTION: appeler immédiatement un CENTRE ANTIPOISON ou un médecin. **P305+P351+P338** EN CAS DE CONTACT AVEC LES YEUX: rincer avec précaution à l'eau pendant plusieurs minutes. Enlever les lentilles de contact si la victime en porte et si elles peuvent être facilement enlevées. Continuer à rincer. **P310** Appeler immédiatement un CENTRE ANTIPOISON ou un médecin.

Conditions of selection:

Most cells growing aerobically are killed by Hygromycin B in the concentration range of 10 to 1000 µg/ml. However, the sensitivity of cells is pH dependent, i.e., the higher the pH of culture medium, the greater 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 decrease the amount of Hygromycin B needed.

***Escherichia coli*:** Hygromycin-resistant transformants are selected in Low Salt LB agar medium (yeast extract 5 g/l, tryptone 10 g/l, NaCl 5 g/l, agar 15 g/l, pH 8) supplemented with 50 µg/ml of Hygromycin B. Plates containing Hygromycin B are stable for 1 month when stored at 4 °C

Mammalian cells: The working concentration of Hygromycin B for mammalian cell lines varies from 50 to 1000 µg/ml. In a starting experiment we recommend to determine the optimal concentration of Hygromycin B required to kill your host cell line. Killing and detachment of dead cells from the plate, especially at high cell density, can require a longer time than with G418. Hygromycin-resistant stable transfectants are usually obtained after 10 days to 3 weeks incubation, depending on the cell line. Suggested concentrations of Hygromycin B for selection in mammalian cells are listed below:

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

Hygromycin B is suitable for research only. It must not be used on humans