



Hygromycin B Antibiotic Solution

(Research Use Only, Not for Drug Use)

Cat#	Product Name	Amounts
<u>Hygro-anti</u>	Hygromycin B solution in PBS, cell culture ready	1.0 ml (50 mg/ml)

Storage: Upon received, it should be stored at 4°C-20°C. Stable for 6 months.

Product Description:

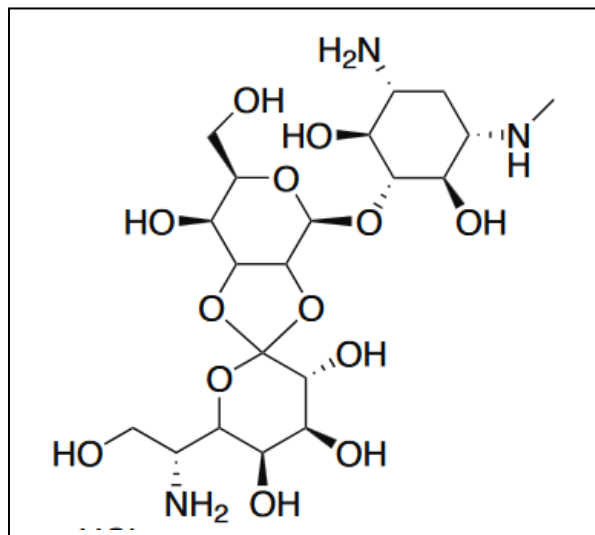
Hygromycin B is produced by *Streptomyces hygroscopicus*. It inhibits polypeptide synthesis by binding to the 80S subunit, therefore, inhibiting translation. Resistance to HYGROMYCIN B is conferred by a kinase that inactivates hygromycin B through phosphorylation.

It is used in cell biology as a selective agent to select transformed cells which have been engineered to the hygromycin-resistance gene.

It is toxic to prokaryotic and eukaryotic cells, thus used as a selection maker for Hygromycin-resistant transforms in mammalian cells, plant cells, or bacteria at its working concentration.

Specification:

- 1) 0.22ul filter sterilized solution in PBS at 50 mg/ml stock, Cell culture tested.
- 2) Compound name: Hygromycin B
- 3) CAS number: 31282-04-9
- 4) Cell-culture tested: toxicity and potency validated mammalian cell lines.
- 5) Formula: $C_{20}H_{37}N_3O_{13}$
- 6) Molecular weight: 527.53 g/mol
- 7) Safety consideration: this solution is provided for stable cell selection, and for research use only, not for drug use, not for animal diagnostic or therapeutic use. Please refer to its MSDS file for handling instructions.
- 8) Structure:



Working Concentrations:

The working concentration of antibiotic is dependent cell types and antibiotic's potency. It must be obtained from the killing curve test in your specific cell type. The optimal concentration is the minimal concentration that kill all your cells in the define time-course. Hygromycin B kills eukaryotic cells in about 2 to 3 weeks.

The working concentration of Gentarget's Hygromycin B solution were tested in some mammalian cell types against Gentarget's lentivirus products containing Hygromycin resistant gene.

You can use the working concentration listed below for the matched cell type.

Working Concentration of Gentarget's Antibiotic Solution.

Cell Line	Hygromycin B (final CONC in medium, ug/ml) * (CAT#: Hygro-anti)
CHO	250
HeLa (Human)	500
HT1080	400
Hek293 (Human)	400
Jurkat T (Human)	1000
MCF10A cell (Human)	200
MCF-7 (Human)	400



*: Note: The working concentration above is provided as reference. You may test the killing curve for your cell culture conditions using following protocol.

Antibiotic Selection protocol:

Day 0:

Seed cells in complete medium at the appropriate density and incubate overnight in 24 well/plate.

Note: at the time of selection, cells should be 50%-75% confluent.

Day 1:

- ☐ Thaw Hygromycin B solution at 37°C. Under biological hood (sterilized condition), mix via pipetting to make sure it is in fully clear solution, no pellet left.
- ☐ Make series dilution of Hygromycin B from 10 ug/ml to 1000 ug/ml final concentration in your complemented medium.
- ☐ Remove the culture medium from the cell wells, and add 0.5 ml/per well of Hygromycin B containing medium (with the series diluted concentration) at each well.
- ☐ Return cells to grow in incubator.

Day 10~21:

Observe the cell death (detached or shirked) in each well. The optimal concentration is the minimal concentration that kill all cells in that well.

Then, use this optimal concentration to select your Hygromycin B-resistant stable cells in target wells where a Hygromycin B resistant plasmid was transfected. Remember to set up the controls without transduction where all cells should be died after the selection.

Safety Precaution:

This antibiotic solution is provided for research use only, not for drug use, or clinical use. Please refer to MSDS file for handling this harmful material.