

Technical Information

Hygromycin B

With 50 mg/ml Hygromycin B in sterile tissue culture grade water

Product Code: A01015

Application:-

Hygromycin B is an aminoglycoside antibiotic isolated from *Streptomyces hygroscopicus*. It inhibits the growth of prokaryotic and eukaryotic cells. Specifically, it inhibits protein synthesis by interfering with translocation of the 70S ribosome subunit and inducing misreading of the mRNA template. Hygromycin B has been used to select transfectants in a wide variety of cells including bacteria, protozoans, yeast, fungi, plants, and mammalian cells. Resistance to Hygromycin B is conferred by a gene coding for a phosphotransferase (hpt or hph) that phosphorylates Hygromycin B, thereby inactivating it. Therefore is an effective agent for the selection and maintenance of drug-resistant stable transfectants.

Methodology

Recommended concentration for the selection of resistant cells is 25-1000µg/ml of Hygromycin B powder or 0.125- 5ml/L of A01015. However, commonly used concentrations for selection of mammalian, plant, bacteria cells and fungi are given in the table below

Species	Cell line	Hygromycin B	
		µg/ml	ml/L
<i>E.coli</i>	JM 83	25-100	0.5 - 2
<i>Streptomyces lividans</i>		50	1
<i>Saccharomyces cerevisiae</i>		200	4
<i>Aspergillus nidulans</i>	GR 1	250	5
<i>Aspergillus nidulans</i>	GR 5	250	5
	G 191	250	5
	G 20	750	15
	W 1	> 1000	> 40
	FGSC 4	> 1000	> 40
	FGSC 237	> 1000	> 40
Tobacco		20 - 200	0.4 - 4
Chicken	DT 40	1500	30
	27 C 2	1500	30
	RP 9	1500	30
	HD 3	1500	30
	CU 39	1500	30
	BM 2	1500	30

Species	Cell line	Hygromycin B	
		µg/ml	ml/L
Mouse	LTK	200 - 400	4 - 8
	L 929	200	4
	C 127	300	6
	NIH 3T3	50 - 500	1 - 10
	PA 317	50	1
Rat		200	4
Mink	CCL 64	400	8
Human	B 95-8	50	1
	Raji	100	2
	721	100	2
	WIL 2 TK	200	4
	Daudi	200	4
	GG 68	400	8
	293	200	4
	K 562	200	4
Mouse-Human	SCC 16-5	200	4

The cells are transfected with gene coding for a phosphotransferase enzyme (hpt or hph) and are incubated in a regular growth medium containing Hygromycin B to select for stable transfectants. Note that the cells can escape the selection if the antibiotic is used at too low concentrations or if the plating density is too high. For the selection of transfected Hygromycin B resistant cells, a concentration of Hygromycin B should be used, that completely blocks the growth of sensitive, non-transfected cells.

Procedure for selection of transfected cells:

1. Remove culture medium and add 5-6ml fresh culture medium, containing Hygromycin B to 60mm culture dish containing the freshly transfected cells.
2. After 5-7 days replace medium by fresh culture medium containing Hygromycin B, if required.
3. Further incubate cells for 5-7 days.
4. After this incubation period the cell cultures will contain only living cells expressing the Hygromycin B resistant phenotype. Therefore culture medium containing Hygromycin B may be replaced by fresh culture medium, but without addition of Hygromycin B.
5. Depending on transfection efficiency and proliferation kinetics of the cells being transfected, it may be necessary to subculture adherent cells.
6. After successful transfection and selection, Hygromycin B resistant transfectants may be cloned.

Quality control

Appearance

Brown colored, clear solution

pH

9.00 - 11.00

Osmolality

80 - 120 mOsm/ KgH₂O

Hygromycin B concentration

50mg/ml

Sterility

No bacterial or fungal growth was observed after 14 days of incubation as per USP specification

Cultural Response

No toxicity to cells

Antibiotic sensitivity test

Passes

Endotoxin Content

NMT 0.5 EU/ml

Storage and Shelf Life

Store at 2-8°C away from bright light.

Shelf life is 18 months.

Use before expiry date given on the product label.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conforms solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate.
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