Ver. HB230112

Hygromycin B

Product description

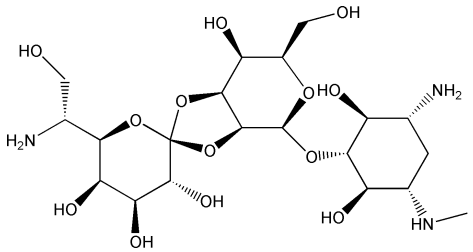
Hygromycin B, an aminoglycoside antibiotic synthesized by Streptomyces hygroscopicus, inhibits protein synthesis by interfering with 70S ribosomal translocation and inducing misreading of mRNA template, thus killing prokaryotes (such as bacteria), eukaryotes (such as yeasts, fungi) and higher mammalian eukaryotes.

Hygromycin resistance genes (hyg or hph) derived from Escherichia coli encode hygromycin B phosphotransferase, which detoxifies hygromycin B into a non-biologically active phosphorylated product, Imaking it a very useful selective marker for screening and culturing prokaryotic or eukaryotic cells successfully transfected with hytromycin resistance genes. In addition, due to different modes of action, Hygromycin B is often used in combination with G418 or Blasticidin S for selection of dual-resistant positive cell lines. Hygromycin B can also be used as an antiviral agent because it selectively penetrates into cells with increased membrane permeability due to viral infection and has the effect of inhibits translation. It can also act as insect-repellant via mixed with animal feed.

Components

Components No.	Name	60224ES03	60224ES10
60225	Hygromycin B	1 g	10 g

Specifications

CAS No.	31282-04-9
Molecular formula	$C_{20}H_{37}N_3O_{13}$
Molecular weight	527.52 g/mol
Purity	>90% (HPLC)
Potency	⩾1050 U/mg
Structure	

Storage

The product should be stored at -25°C ~ -15°C for two years.

Instructions

1. Commonly used screening concentration

Generally, mammalian cells: 50-500 μg/mL; Bacterial/plant cells: 20-200 μg/mL; Fungi: 300-1000 μg/mL.

Establishment of killing curve

Note: In order to screen stable cell lines, it is necessary to determine the minimum concentration of antibiotics capable of killing untransfected host cells. This can be achieved by establishing a killing curve (dose-response curve). At least five concentrations should be arranged.

1.1 Day 1: Untransformed cells are planked in an appropriate culture plate at a cell density of 20-25% and cultured overnight.

Note: The amount of inoculation cells can be increased for cells requiring higher density to detect vitality.

1.2 Set the concentration gradient within the appropriate range according to the cell type. Mammalian cell can set 50, 100, 250, 500, 750, 1000 μg/mL. Dissolve hygromycin B powder with PBS and prepare a 50 mg/mL solution. Dilute the Hygromycin B solution 1:10 with deionized water or PBS buffer to 5 mg/mL. And then dilute the solution to the corresponding working concentration according to the following table.

Final Concentration (μg/mL)	Medium Volume (mL)	Addition Volume of 5 mg/mL Hygromycin B (mL)
50	9.9	0.1
100	9.8	0.2
250	9.5	0.5
500	9.0	1.0
750	8.5	1.5
1000	8.0	2.0

1.3 Day 2: Replace with a freshly prepared medium containing the corresponding concentration of the drug. Make three parallel samples for each concentration.

1.4 Then replace with fresh media containing drugs every 3-4 days.

1.5 Living cell counts are performed at a fixed cycle (e.g., every 2 days) to determinee working concentration of hygromycin B for screening stable strains varies according to cell type, culture medium, growth conditions and cell metabolic rate. It is recommended to establish a kill curve (dose-response curve) to determine the optimal screening concentration for the first time. And the recommended working concentration is 50-1000 μg/mL.

The appropriate concentration. Choose the minimum concentration which kills the majority of cells within an ideal number of days (usually 7-10 days), as the working concentration for screening stable cell line.

2. Screening of stable cell lines

The effective screening concentration of purinomycin is related to cell type, growth state, cell density, cell metabolism and cell cycle position. To screen for stably expressing shRNA cell lines, it is critical to determine the minimum concentration of puromycin that kills untransfected/transduced cells. It is recommended that customers who are doing experiments for the first time must establish a kill curve suitable for their own experimental system.

2.1 Day 1: The 24-well plate is plated at a density of $5\sim8\times10^4$ cells/well, and a sufficient number of wells are plated for subsequent gradient experiments. Cells were incubated overnight at 37°C.

2.2 Day 2: A) Prepare screening medium: fresh medium containing different concentrations of puromycin (such as 0-15 μg/mL, at least 5 gradients); B) Replace the freshly prepared screening medium in the cells after overnight incubation; Then the cells are incubated at 37°C.

2.3 Day 4: Replace with fresh selection medium and observe cell viability.

2.4 Depending on the growth state of the cells, change to fresh selection medium every 2-3 days.

2.5 Cells were monitored daily to observe the rate of viable cells to determine the lowest concentration of drug effective to kill non-transfected or all non-transduced cells within 4-6 days of the start of antibiotic screening.

3. Screening of Mammalian Stably Transfected Cell Lines

3.1 48 h after transfection, the cells were subcultured by screening medium containing hygromycin B at appropriate concentration (direct or diluted).

Note: Antibiotics work best on actively dividing cells. If the cells are too dense, the antibiotic will not kill the cells. Split the cells such that the cells are no more than 25% confluent.

3.2 Change the screening medium every 3-4 days.

3.3 Measure the cell colony-formation after 7 days of screening. Colony formation may take another week or more, depending on host cell type, transfection, and screening effectiveness.

3.4 Pick 5-10 resistant clones and transfer to 35 mm cell culture plates, and cultured with drug-containing screening medium for 7 days.

3.5 Replace with fresh medium without drugs for culture.

Notes

1. Hygromycin B resistance gene (hyg or hph), except from E. Coli., are also found in other bacterial strains, including Streptomyces hygroscopicus and Klebsiella pneumoniae.
2. Toxic compound, avoid skin and eyes contact, please handle with care.
3. For your safety and health, please wear lab coats and disposable gloves for operation.
4. For research use only.