Hygromycin B

V02

Cat: H8080
Specification:10mL (1g)
Storage: Store at 2-8℃, and it is valid for 4 years.

Product Information
CAS： 31282-04-9
English name: Hygromycin B
Appearance (Character): Colorless to light-yellow transparent liquid
Molecular Formula: $C_{20}H_{37}N_3O_{13}$
Molecular Weight: 527.52
Concentration: 100mg/mL
Enzyme activity/potency: 1100 units/mg
Danger code: R: 26/27/28-41-42/43 S: 22-26-28-36/37/39-45

Introduction

Hygromycin B is an aminoglycoside antibiotic produced by Streptomyces hygroscopicus. It inhibits protein synthesis by interfering with 70S ribosome translocation and inducing misreading of mRNA templates, thereby killing prokaryotic organisms such as bacteria, eukaryotic organisms such as yeast, fungi, and higher mammalian eukaryotic cells. It is a protein synthesis inhibitor that can be used in biochemical research such as plant cell culture.

The hygromycin resistance gene hyg or hph from Escherichia coli. encodes hygromycin B phosphotransferase, which converts hygromycin B into a non-biologically active phosphorylated product, thereby playing a detoxifying role. In light of this principle, hygromycin B is a highly effective selective marker for screening and maintaining prokaryotic or eukaryotic cells that have been successfully transfected with hygromycin resistance genes. In addition, due to the difference in mode of action, it is often used in combination with G-418 (Cat:G8161), Zeocin(Cat:Z8020), and Blasticidin S(Cat:B9300) for the selection of double-resistant positive cell lines. Hygromycin B can also be used as an antiviral agent because it selectively penetrates into cells with enhanced permeability due to viral infection and has the effect of inhibiting translation. It can also be mixed into animal feed to have an insect repellent function.

Instructions for use(*for reference only*):

This product is a sterile hygromycin B solution at 100mg/ml, which can be directly diluted with culture medium for use.

1. Common screening concentration

Note: The working concentration of hygromycin B used to screen stable strains needs to vary depending on cell type, culture medium, growth conditions, and cell metabolic rate. The recommended concentration is 50-1000 μg/mL. For the first time using the experimental system, it

is recommended to establish a kill curve, or dose-response curve, to determine the optimal screening concentration.

Generally speaking, mammalian cells are 50-500μg/mL; Bacterial/plant cells 20-200μg/mL; Fungus 200-1000μg/mL.

2. Establishment of the kill curve

Note: In order to screen cell lines that stably express the target protein, it is necessary to determine the minimum concentration of antibiotics that can kill untransfected host cells. This can be achieved by establishing a dose-response curve for killing, selecting at least five concentrations.

1) Day 1: Untransformed cells are seeded at a cell density of 20-25% on suitable culture plates, incubated at 37°C with CO_2 overnight; Note: For cells that require higher density to detect viability, the inoculation volume can be increased.

2) Set a concentration gradient within an appropriate range according to the cell type. Taking mammalian cells as an example, concentrations of 50, 100, 250, 500, 750, and 1000 μg/mL can be set. First, dilute the stock solution to 5 mg/ml with deionized water or PBS buffer at a ratio of 1:10, and then dilute it to the corresponding concentration of working solution according to the following table.

Final concentration (μg/mL)	Volume of culture medium (ml)	5mg/ml hygromycin B added (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

3) Day 2: Replace the old culture medium with freshly prepared culture medium containing the corresponding concentration of drug. Three parallel wells were prepared for each concentration.

4) Next, replace the new drug-containing medium every 3-4 days.

5) Conduct live cell counting at fixed intervals, such as every 2 days, to determine the appropriate concentration to prevent the growth of untransfected cells. The minimum concentration that can kill the majority of cells within 7-10 days is usually selected as the working concentration for stable transfection cell screening.

3. Selection of Stably Transfected Cells

1) After 48 hours of transfection, use a selection medium containing appropriate concentrations of hygromycin B to pass on the cells directly or after dilution.

Note: Antibiotics are most effective when the cells are actively dividing. When the cells are too dense, their efficiency will decrease. To achieve better screening results, it is best to dilute the cells to an abundance of no more than 25%.

2) Replace the screening medium containing drugs every 3-4 days.

3) After 7 days of screening, the formation of cell clone colonies was observed and evaluated. The formation of colonies may take another week or more, depending on the type of host cells, transfection, and screening efficiency.

4) Pick and transfer 5-10 resistant clones to a 35mm cell culture plate, and continue to maintain the culture with drug-containing selection medium for 7 days.

5) Replace the normal culture medium and culture it.

Note

1. The hyg or hph resistance gene of hygromycin B is derived from E Besides Coli, it has also been found in other strains, including Streptomyces hygroscopicus and Klebsiella pneumoniae.
2. This product is a toxic compound that is irritating to the eyes and skin. Wear protective gear and operate carefully with local ventilation.
3. The product information is for reference only. If you have any questions, please call 400-968-6088 for consultation.
4. The products are all for scientific research use only. Do not use it for medical, clinical diagnosis or treatment, food and cosmetics, etc. Do not store them in ordinary residential areas.
5. For your safety and health, please wear laboratory clothes, disposable gloves and masks to operate.

Q and A:

1: How can non-transfected cells escape antibiotic selection?

Cells can escape selection if the antibiotic is used at too low concentration or if the cell density on the plate is too high. Additionally, cells rapidly proliferating are killed faster than those proliferating slowly. Control cells should die within 5-7 days after addition of the antibiotic allowing colonies of resistant cells to form by 10-14 days.

2: How do I determine the Toxic Concentration?

Hygromycin B is added to the culture medium at a concentration that varies with the cell type transfected. A titration experiment for each cell type may therefore be performed to determine the amount of Hygromycin B needed to kill non-transfected cells. The working concentration for mammalian cell selection is normally between 50ug/ml and 1mg/ml, Plant cells: 20-200ug/ml, Bacteria: 20-200ug/ml and Fungi: 200ug-1mg/ml. Your appropriate concentration should be tested experimentally.

3: How do I perform a Dose Response curve?

To determine the minimum concentration of antibiotic required to kill your non-transfected host cell line.

1) Test arrange of concentrations to ensure that you determine the minimum concentration necessary for your cell line.

2) Seed cells at approxiamately 20-25% confluency on the appropriate number of plates for each time plate and allow cells to adhere overnight. For cells that require higher densities for viability, increase the number of cells seeded.
3) The next day, substitute culture medium with medium containing varying concentration of the antibiotic.
4) Replenish the selective medium every 3-4 days.
5) Count the number of viable cells at regular intervals to determine the appropriate concentration of antibiotic that prevents the growth of non-transfected cells. Select the concentration that kills the majority of the cells in the desired number of days, usually 7-10 days.

4: How do I maintain Hygromycin resistant phenotype of transfected cell lines?

To maintain Hygromycin resistant phenotype of transfected cell lines and for the elimination of revertants cells may be regularly cultured in culture medium containing Hygromycin B at the same concentration used for the initial selection.

5: Replacement of Media?

Replacement of culture media containing Hygromycin B is needed only if nutritional components are consumed by the cells cultured. Acidification of the culture medium is a normally a sign of consumption. Utilizing phenol red or media containing phenol red will aid in the detection of acidification. In this case the media will turn yellow.

6: Is hygromycin B sensitive to acid?

Hygromycin B is sensitive to high concentrations of acid, but brief exposure to dilute acid does not affect its stability.

7: Can we increase the sensitivity of cells to antibiotics?

The sensitivity of cells to hygromycin B can be increased by increasing the pH value of the culture medium. The lower the salt concentration, the higher the sensitivity.

8: What enzyme can inactivate hygromycin B?

Hygromycin phosphotransferase (hpt) inactivates antibiotics through phosphorylation. The hygromycin phosphotransferase gene (hpt, hph or aphIV) encodes hygromycin phosphotransferase and is used as a selectable marker gene in plant and animal systems.