潮霉素B(液体)(100mg/mL)

CAT NO： K10001
CAS: 31282-04-9
APPEARANCE: Clear and yellowish solution
CONCENTRATION: 100mg/ml in water
STORAGE: Store at +4°C. PROTECT FROM LIGHT!

Caution: Hygromycin B is extremely irritating to the eyes and moderately irritating to the skin. Absorption through the eyes and skin can have a toxic effect. Prevent contact and inhalation. Wear respirator, paper jacket and pants, head covering and rubber gloves when handling this material. Use local exhaust ventilation.

Description: Hygromycin B, an aminoglycosidic antibiotic produced by Streptomyces hygroscopicus , is used for the selection and maintenance of prokaryotic and eukaryotic cells transfected with the hygromycin resistance gene, hph. Hygromycin B kills bacteria, fungi and higher eukaryotic cells by inhibiting protein synthesis. The resistance gene codes for a kinase that inactivates Hygromycin B through phosphorylation. Cloning of the resistance gene and fusion with eukaryotic promoters has resulted in the development of vectors that permit selection for resistance to Hygromycin B in both prokaryotic and eukaryotic cells. The working concentration for the purpose of selection varies with cell type, media, growth conditions and cell metabolic rate. Recommended concentration for the selection of resistant cells is 25-1000ug/ml. Commonly used concentrations for selection are 200ug/ml for mammalian cells, 20-200ug/ml for plant cells & bacteria cells and 200-1000ug/ml for fungi. Your optimum concentration should be tested experimentally.

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

ANSWER: 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.

QUESTION: How do I determine the Toxic Concentration?

ANSWER: 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.

QUESTION: How do I perform a Dose Response curve?

ANSWER: To determine the minimum concentration of antibiotic required to kill your non-transfected host cell line. Test arrange of concentrations to ensure that you determine the minimum concentration necessary for your cell line. 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. The next day, substitute culture medium with medium containing varying concentration of the antibiotic. Replenish the selective medium every 3-4 days. 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.

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

ANSWER: 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.

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QUESTION: Replacement of Media?

ANSWER: 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.

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