Puromycin dihydrochloride hydrate

V02

Cat: P8230
Specification:25mg /100mg
Storage: Store at -20° C, and it is valid for 4 years.

Product Information
CAS: 58-58-2
English name： Puromycin dihydrochloride hydrate
Alias: Puromycin dihydrochloride
Appearance (Character): White to light yellow powder
Molecular Formula： $C_{22}H_{29}N_7O_5 \cdot 2HCl$
Molecular Weight: 544.44
Purity: ≥99%
Solubility: 50 mg/mL in water
Hazard Code: R:22

Introduction

Puromycin belongs to the amino-nucleoside family of antibiotics and is isolated from Streptomyces alboniger. Since the partial structure of this antibiotic showed it to be a purine derivative, puromycin was assigned as its generic name.

Puromycin is a broad spectrum antibiotic and antibacterial agent. It is active against Gram-positive microorganisms, less active against acid-fast bacilli, and weakly active against Gram-negative microorganisms.It acts very quickly and can kill 99% of the cells within 2 days. It also exhibits antitumor activity in studies on brain tumor cells.

Puromycin is a protein synthesis inhibitor that causes premature chain termination by acting as an analog of the 3'-terminal end of aminoacyl-tRNA. It has been used to study transcriptional regulatory mechanisms that control the sequential and coordinate expression of genes during cell differentiation.

Protocols(*for reference only*):

1. Recommended Concentrations

Mammalian cells: 1-10 μg/mL. The optimal concentration should be determined by a kill curve.

Escherichia coli: For screening stable transformants carrying the pac gene in LB agar plates, use a concentration of 100-125 μg/mL. Note: Precise pH adjustment is necessary for puromycin selection in E. coli, and the host cell itself can influence the selection. (The working concentration of puromycin should be adjusted appropriately based on cell type, medium, growth conditions, and cellular metabolic rate.)

2. Dissolution Method

Dissolve puromycin in distilled water to prepare a stock solution of 50 mg/mL. Filter sterilize through a 0.22 μm filter and aliquot for storage at -20°C. Alternatively, dissolve in methanol to prepare a 10 mg/mL stock solution.

3. Determination of the Puromycin Kill Curve (Example using shRNA transfection or lentiviral transduction)

The effective screening concentration of puromycin is related to cell type, growth status, cell density, cell metabolism, and the position of cells in the cell cycle. Determining the lowest concentration of puromycin that kills untransfected/untransduced cells is crucial for selecting stably expressing shRNA cell lines. It is recommended that new users establish a kill curve suitable for their experimental system.

1) Day 1: Seed 24-well plates at a density of 5-8 × 10^4 cells/well. Incubate the cells overnight at 37°C.
2) Day 2: a) Prepare screening medium: fresh medium containing different concentrations of puromycin (e.g., 0-15 μg/mL, at least five gradients). b) Replace the overnight incubated cells with freshly prepared screening medium. Incubate the cells at 37°C.
3) Day 4: Replace the screening medium and observe the survival rate of the cells.
4) Every 2-3 days: Based on the growth status of the cells, replace the screening medium.
5) Daily monitoring: Observe the survival rate of the cells to determine the minimum concentration of antibiotic that effectively kills non-transfected/non-transduced cells within 4-6 days of starting the selection.

4. Selection of Stable Transfected Mammalian Cell Lines

After transfecting cells with plasmids containing the pac gene, grow the cells in medium containing puromycin to select for stable transfectants.

1) 48 hours post-transfection: Seed the cells (undiluted or diluted) in fresh medium containing the appropriate concentration of puromycin. Note: Antibiotics are most effective when cells are actively dividing. If cells are too dense, the effectiveness of the antibiotic will significantly decrease. It is best to split the cells to maintain a density of less than 25%.
2) Every 2-3 days: Remove and replace the medium containing puromycin.
3) After 7 days: Evaluate the colonies formed by the cells. Colonies may require an additional week or more, depending on the host cell line and the efficiency of transfection selection. Note: Monitor the growth status of the cells daily. Puromycin selection requires at least 48 hours, and the screening period with an effective concentration of puromycin generally lasts 3-10 days.
4) Transfer and place 5-10 resistant clones into a 35 mm dish, and continue to culture them in selective medium for 7 days. This enrichment culture is preparatory for subsequent cytotoxicity assays.

Note

1. Puromycin is a toxic compound. Handle with care when using it.
2. Unless otherwise specified, the biochemical reagents produced by our company are generally non-sterile packaged. If they are to be used for cell experiments, please conduct pretreatment in advance.
3. Once dissolved, please store the solution in separate containers to avoid product degradation caused by repeated freezing and thawing.
4. The product information is for reference only. If you have any questions, please call 400-968-6088 for consultation.
5. 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.
6. For your safety and health, please wear laboratory clothes, disposable gloves and masks to operate.