Puromycin

Cat: P8231
Specification: 1mL /10*1mL /50mL
Storage: Short-term storage at 4°C, long-term storage at -20°C.

Product Information
CAS： 58-58-2
English name： Puromycin
Appearance (Character)： Liquid
Molecular Formula： $C_{22}H_{29}N_7O_5 \cdot 2HCl$
Molecular Weight： 544.44
Purity: ≥98%

Introduction

Puromycin, an aminoglycoside antibiotic produced by the fermentation metabolism of Streptomyces alboniger, kills Gram-positive bacteria, various animal and insect cells by inhibiting protein synthesis. It is also effective against Escherichia coli in some special cases. The mechanism of action lies in the fact that puromycin is an analog of the 3' terminus of the aminoacyl-tRNA molecule, which can bind to the A site of the ribosome and incorporate into the extended peptide chain. After binding to the A site, puromycin will not participate in any subsequent reactions, leading to the premature termination of protein synthesis and the release of immature polypeptides with puromycin at the C-terminus.

The pac gene found in the puromycin-producing bacterium Streptomyces alboniger encodes puromycin N-acetyltransferase (PAC), which confers resistance to puromycin. This characteristic is now widely used to screen for stable transfected mammalian cell lines that specifically carry the pac gene plasmid.

The ubiquitous application of puromycin in the screening of stable cell lines is related to the properties of lentiviral vectors, as most commercialized lentiviral vectors currently carry the pac gene. In some specific cases, puromycin can also be used to screen for Escherichia coli strains transformed with pac gene-carrying plasmids.

Instrustions of use:

1. Recommended concentration

Mammalian cells: 1-10 μg/mL, the optimal concentration needs to be determined by the killing curve;

Escherichia coli: LB agar medium is used to screen stable Escherichia coli transformed with pac gene, with a concentration of 125μg/mL.

Note: The use of puromycin to screen stable E. coli strains requires precise pH adjustment and is influenced by the host cell itself.

2. Determination of the killing curve of puromycin with shRNA transfection or lentiviral transduction as an example

The effective screening concentration of puromycin is related to cell type, growth status, cell density, cell metabolism, and the position of the cell in the cell cycle. To select stable shRNA cell lines, it is crucial to determine the minimum concentration of puromycin that can kill untransfected/transduced cells. It is recommended that customers who are conducting experiments for the first time must establish a kill curve that is suitable for their own experimental system.

1) Day 1:4 5~8×10 4 cells/well were placed in the orifice plate, and enough wells were placed for subsequent gradient experiments. Incubate the cells at 37℃ overnight;

2) Day 2: Prepare screening medium: fresh medium containing different concentrations of puromycin, such as 0-15μg/mL, with at least 5 gradients; b. After overnight incubation, replace the freshly prepared screening medium in the cells; After that, incubate the cells at 37℃;

3) Day 4: Replace the fresh screening medium and observe the cell survival rate.

4) Depending on the growth status of the cells, replace the fresh screening medium with fresh one every 2-3 days;

5) Monitor cells daily and observe the survival rate of cells to determine the minimum concentration of drugs that can effectively kill non-transfected or all non-transduced cells within 4-6 days of starting antibiotic screening.

3. Screening of stable transfected cell lines in mammals

After transfecting the plasmid containing the pac gene, the cells are propagated in a medium containing puromycin to select stable transfectants.

1) After 48 hours of transfection, the cells were cultured as they were or diluted and placed in fresh medium containing appropriate concentrations of puromycin.

Note: Antibiotics are most effective when cells are in the active stage of division. The efficacy of antibiotics will significantly decrease when the cells are too dense. It is best to divide the cells into plates so that the density does not exceed 25%.

2) Remove and replace the culture medium containing puromycin every 2-3 days.

3) After 7 days of screening, the lesions formed by the cells were evaluated. The focus may require an additional week or more, depending on the host cell line and transfection screening efficiency. Note: Observe the growth status of cells daily. The screening of puromycin requires at least 48 hours, and the screening cycle for effective concentrations of puromycin is generally 3-10 days.

4) Transfer and place 5-10 resistant clones into a 35mm culture dish, and continue to culture for 7 days using selection medium. The enrichment culture is to prepare for the cytotoxicity experiment in the future.

Note

1. 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.
2. Once dissolved, please store the solution in separate containers to avoid product degradation caused by repeated freezing and thawing.
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.