Puromycin (10 mg/mL)

Cat. No.: PB180132

Size: 1mL / 1mL×10

Product Description

Puromycin is a kind of aminoglycoside antibiotic produced by the fermentative metabolism of Streptomyces albicans.

The mechanism of action of puromycin is to inhibit peptidyl transfer on prokaryotic and eukaryotic ribosomes; the pac gene found in Streptomyces alboniger encodes puromycin N-acetyltransferase (PAC), which confers resistance to puromycin. This feature is now commonly used in the selection of specific mammalian stably transfected cell lines carrying pac gene plasmids.

General Information

Form	Liquid
Concentration	10 mg/mL
Recommend Working Concentration	1-10 μg/mL
Size	1mL/1mL×10
Solvent	Ultrapure water
Storage Conditions	-5~-20°C. Protect from light
Transport Conditions	Ice bag
Expiration Date	12 months

Protocol

1. Recommended Working Concentration

Recommended concentration of puromycin hydrochloride for some cell lines (optimal concentration requires a kill curve to determine):

Cell Line	Concentration
B16	1-2 μg/mL
HEK293	0.5-10 μg/mL
HeLa	1-10 μg/mL
MEF	1-5 μg/mL
HepG2	0.5-5 μg/mL
A549	1-2 μg/mL
H1299	1-3 μg/mL
HT1080、MCF-7	0.5-2 μg/mL
MDA-MB-231	0.5-5 μg/mL

2. Determination of puromycin killing curve (using lentivirus transfection as an example)

The effective screening concentration of puromycin is related to cell type, growth state, cell density, cell metabolism and cell cycle position.

In stable transfection, it is usually necessary to determine the lowest puromycin concentration that can kill untransfected cells by consulting the literature or experimental determination in advance. This concentration can not only ensure that the dose of puromycin added during subsequent stable rotation screening has a sufficient killing effect on untransfected cells, but also ensure the minimum toxicity to

transfected cells. It is suggested that customers who do experiments for the first time should establish a kill curve suitable for their own experimental system.

1) Day 1: Plate at a density of 5-8×10^4 cells/well in 24-well-plate and culture overnight at 37°C.
2) Day 2: ①Prepare drug-containing medium: fresh medium containing puromycin with different Concentration gradients (e.g. 0-15 μg/mL, at least 5 gradients); ②Replace each well with each gradient drug-containing medium, and continue to culture cells at 37°C.
3) Day 3-6: Replace the fresh drug-containing medium every 48 h, continuously culture the cells, and observe the cell survival rate.

The cells were monitored daily to observe the rate of viable cells and determine the lowest drug concentration effective to kill non-transfected cells within 4-6 days of dosing.

3. Stable transfection

After transfection of the resistance gene, the cells are continuously cultured in the medium containing the selected antibiotic, and the stable cell line can be selected after killing the non-transfected cells.

1) After 48 h of cell transfection, the cells were placed in fresh medium containing the appropriate concentration of the selection antibiotic for continuous culture.

Note: When the cell is in the active phase of division, the effect of antibiotics is most obvious. If the cells are too dense, the killing effect of antibiotics will be significantly reduced. It is best to adjust the passage ratio appropriately when passing cells so that the density after passage does not exceed 25%.

2) Every 48-72 h, replace the fresh screening medium.
3) The cell lines can be identified 7 days after the start of the screening to determine whether the stabilization screening is complete. Stabilization screening usually lasts for 1 to 2 weeks or more, and the length of time is related to the host cell line and transfection efficiency.
4) After the screening is completed, the species can be passaged and expanded and frozen and stored as needed.

Once the stable transfection cell line is successfully established, a lower concentration of screening antibiotics can be continuously added according to their own needs in the subsequent culture process to maintain the stability of its purity, and 50% of the screening concentration is usually selected as the maintenance concentration.

Notes

1. This product is only used for scientific research or further research, not for diagnosis and treatment.
2. Puromycin is a toxic compound, please do a good job of relevant protection during operation.
3. This product has been filtered and sterilized by 0.1 μm filter, can be used directly after melting.
4. When using this product, attention should be paid to aseptic operation to avoid pollution.
5. The product should be thawed in 2-8°C, shake well after use, avoiding repeated freezing and thawing.
6. If there are precipitates after thawing, they can be vortexed and mixed evenly or blown with a pipette. After standing at room temperature for about 1 hour or at 37°C in an incubator for 20-30 minutes, observe whether the precipitates can be dissolved normally, and if they can be dissolved, it can be used normally.
7. This product is a concentrated liquid, please dilute it as needed.
8. It is recommended to use the regular at 2-8°C for preservation within one month. It needs to be frozen at -5~-20°C when not in use for a long time, and it is not suitable for long-term storage at room temperature or 2-8°C. To avoid repeated freezing and thawing, it is recommended to store it in small quantities after subpackaging.