Blasticidin S (10 mg/mL)

Cat. No.: PB180133

Size: 1mL / 1mL×5

Product Description

Blasticidin S is a kind of peptidyl nucleoside antibiotic isolated from Streptomyces griseochromogenes. It is a potent inhibitor of protein synthesis in bacteria and eukaryotes, while also active against fungi, nematodes, and tumor cells. Blasticidin S acts by blocking hydrolysis of peptidyl-tRNA induced by release factors and inhibits peptide bond formation. It is useful as a selective agent for mammalian cells and bacterial cells.

Blasticidin S is commonly used to select mammalian cell lines that have been stably transfected with the bsr/BSD/bls gene, often labeled as bsr/bsdr/Blastr plasmid. It has a rapid and potent mode of action, and even very low antibiotic concentrations can quickly kill cells that do not carry the resistance gene.

General Information

Form	Liquid
Concentration	10 mg/mL
Recommend Working Concentration	1-50 μg/mL
Size	1mL/1mL×5
Solvent	Ultrapure water
Storage Conditions	-5~-20°C. Protect from light
Transport Conditions	Ice bag
Expiration Date	12 months

Protocol

1. Recommend Working Concentration

Table 1. Recommend concentration of Blasticidin S hydrochloride for some cell lines (optimal concentration requires a kill curve to determine):

Cell Line	Concentration
B16	3-10 μg/mL
HEK293	5-15 μg/mL
HeLa	2.5-10 μg/mL
CHO	5-10 μg/mL
Hep G2	4-5 μg/mL
A549	8-10 μg/mL
MCF-7	2-5 μg/mL
HT1080	5-20 μg/mL
THP-1	8-10 μg/mL

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

The effective screening concentration of Blasticidin S depends on cell type, growth state, cell density, cell metabolism and cell cycle position.

In stable transfection, it is essential to determine the lowest concentration of Blasticidin S that can kill untransfected cells by consulting literature or experimental determination in advance. This concentration can not only ensure that the dose of Blasticidin S added during subsequent stable

rotation screening has sufficient killing effect on untransfected cells, but also ensure the minimum toxicity to transfected cells. It is recommended that first-time experimenters establish a kill curve tailored to their specific 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 2:① Prepare drug-containing medium: fresh medium with Blasticidin S at varying concentrations (e.g. 0 to15 μg/mL, at least 5 gradients); ② Replace 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 observed daily to assess viable cell rates and determine the lowest drug concentration that effectively kills 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 longer, depending on the host cell line and transfection efficiency.
4) After screening, the species can be passaged, expanded, and frozen for storage as needed.

Once the stable transfection cell line has been 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. Blasticidin S is a toxic compound, please ensure proper 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 contamination.
5. The product should be stored in 2-8°C thawed, shake well after use, repeated freezing and thawing is not recommended.
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.