Blasticidin

V02

Cat: B9300

Specification: 10mg /10*10mg

Storage: Blasticidin is shipped at room temperature. Upon receipt it should be stored at 4 °C or -20 °C. Blasticidin is stable for 2 years at -20 °C, 2 years at 4 °C, and 2 weeks at 37 °C. Avoid repeated freeze-thaw cycles.

Product Information

CAS： 3513-03-09

Molecular Formula： $C_{17}H_{26}N_8O_5 \cdot HCl$

Molecular Weight： 458.9

Purity：>95% (HPLC) , Activity is validated using bioassays on bacteria and mammalian cell lines.

Quantity: 1 ml at 10 mg/ml (10 mg) Blasticidin hydrochloride is supplied as 1 ml tubes of a 10 mg/ml colorless solution in HEPES buffer (100% active compound), pH 7.5, filtered to sterility for customer convenience and cell culture tested.

Introduction

Blasticidin is a peptidyl nucleoside antibiotic isolated from the culture broth of Streptomyces griseochromogenes. It specifically inhibits protein synthesis in both prokaryotes and eukaryotes by inhibiting peptide bond formation in the ribosomal machinery. Blasticidin is used to select transfected cells carrying bsr or BSD resistance genes.

Resistance to blasticidin:

Three blasticidin resistance genes have been cloned and sequenced: an acetyl transferase gene, bls from a blasticidin producer strain, and two deaminase genes, bsr gene from Bacillus cereus, and BSD gene from Aspergillus terreus. Both bsr and BSD genes are used as dominant selectable markers for gene transfer experiments in mammalian and plant cells. Although blasticidin was developed as a selection agent for mammalian cells, the bsr and BSD resistance genes can also be used in E. coli.

Selection conditions:

Escherichia coli

E.coli is poorly sensitive to blasticidin, but transformants resistant to blasticidin can be selected on low salt LB agar medium (pH 8) supplemented with 100 µg/ml blasticidin. High pH enhances the activity of blasticidin.

Mammalian cells

The working concentration of blasticidin for mammalian cell lines varies from 1 to 10 µg/ml, in a few cases up to 30 µg/ml. In a starting experiment we recommend to determine optimal concentrations of antibiotic required to kill your host cell line. After treatment, cell death occurs rapidly, as fast as G418 selection, allowing the selection of transfected cells with plasmids carrying the bsr or BSD genes in as little as 7 days post-transfection. A method for the selection procedure in

mammalian cells is described overleaf. Suggested concentrations of blasticidin for selection in some examples of mammalian cells are listed below.

Cell line	Species	Tissue	Medium	Blasticidin
CHO	Hamster	Ovary	DMEM	5-10μg/mL
HEK293	Human	Kidney	DMEM	5-15μg/mL
HeLa	Human	Uterus	DMEM	2.5-10μg/mL
Neuro2a	Mouse	Neuroblastoma	DMEM	30μg/mL
THP-1	Human	Leukemic monocytes	RMPI	10μg/mL

General guidelines:

Successful transfection is influenced by many factors. The health and viability of the cell line, the quality of the nucleic acid used, the transfection reagent, the duration of transfection, and the presence or absence of serum can all play a part. Several methods for transfection of nucleic acids are available, including treatment with DEAE-dextran, calcium phosphate, viruses or cationic lipids, and electroporation. For stable transfection using cationic lipids, we recommend LyoVec™, a lyophilized transfection reagent with low cytotoxicity.

Method (Selection procedure for mammalian cells)

Forty-eight hours after transfection with a plasmid containing the bsr or BSD gene, cells are incubated in their regular growth medium containing Blasticidin to select for stable transfectants. In order to isolate monoclones, the limiting dilution method is used. With this method, cells are seeded at very low densities (with an average of 0.3 cells per well). A protocol for clone selection and expansion is provided below.

1. The day before transfection, determine the number of cells needed for transfection. Seed the cells at the optimal density and leave overnight.
2. On the day of transfection, determine the viability and the amount of cell clumping from a small aliquot of cells using the trypan blue dye exclusion method.Viability of cells must be over 90%.
   Note: For best results, make sure to have a single cell suspension.
3. Prepare plasmid DNA transfection reagent complex.
4. Seed cells in 1 ml of culture medium per well of a 12-well plate.
   Note: With LyoVec™, the transfection can be performed immediately after cell seeding.
5. Add 50 μLof the DNA-transfection reagent complex to each well and mix gently by rocking the plate back and forth.
   Note: Do not add selection antibiotic immediately after transfection, as this will drastically increase mortality. Allow at least 48 hours before adding the selection antibiotic.
6. Prior to each use, bring blasticidin to room temperature and vortex to homogenize.

7. Forty-eight hours post-transfection, pass cells (direct or diluted) in fresh medium containing blasticidin at the appropriate concentration. Ensure that cells are in the exponential growth phase.

Note: Antibiotics work best when cells are actively dividing. If the cells become too dense, the antibiotic efficiency will decrease.

8. Replace the medium with fresh antibiotic-containing medium every 3 days.

9. Once a population of resistant cells has been obtained, clone these cells at a density of 0.3 cells/well in a 96-well plate.

Notes:

The ratio of 0.3 cells/well provides a very low chance of having two cells in the same well.

To obtain a seeding density of 0.3 cells/well, add 200 ml of a cell suspension of ~1.5 cell/ml in antibiotic-containing medium.

10. After 4 days, assess the number of wells with colonies.

Note: To obtain clonal colonies, as a general rule, the number of positive wells should not exceed 30 per plate.

11. After these wells have been identified, verify cell growth every week. It normally takes 3 weeks to obtain sufficient cells, however, this depends on the growth rate of your cells.

12. Expand the selected single-colony wells.

13. Verify gene expression using the appropriate assays.

14. Upon establishing your target monoclonal stable cell line, you can use a lower amount of antibiotic for maintenance.

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.