

# Blasticidin S

Selective antibiotic for the *bsr* or *BSD* genes

Catalog # ant-bl-1, ant-bl-5, ant-bl-5b

For research use only

Version # 04J06-SV

## PRODUCT INFORMATION

### Contents:

Blasticidin S hydrochloride is supplied as 2 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.

- **ant-bl-1:** 5 x 2ml at 10 mg/ml (100 mg)
- **ant-bl-5:** 25 x 2ml at 10 mg/ml (500 mg)
- **ant-bl-5b:** 1 x 50ml at 10 mg/ml (500 mg)

### Storage and stability:

Blasticidin S is shipped at room temperature. Upon receipt it should be stored at 4°C for immediate use or -20°C for long-term storage. Avoid repeated freeze-thaw cycles.

Blasticidin S is stable for at least one year at -20°C or 4°C and 3 months at room temperature.

### Quality control

Purity controlled by HPLC: >95%

Activity controlled by bioassays on bacteria and mammalian cell lines.

## SPECIAL HANDLING

Blasticidin S is a hazardous compound. Avoid contact with eyes, skin and clothes, harmful if swallowed.

## BACKGROUND

Blasticidin S is a peptidyl nucleoside antibiotic isolated from the culture broth of *Streptomyces griseochromogenes*. It specifically inhibits protein synthesis in both prokaryotes and eukaryotes through inhibition of peptide bound formation in the ribosomal machinery.

Blasticidin S is used to select transfected cells carrying *bsr* or *BSD* resistance genes.

## CHEMICAL PROPERTIES

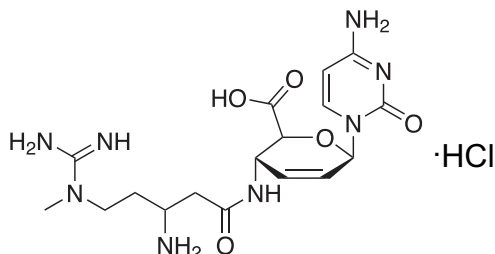
**CAS n°:** 3513-03-9

**Formula:** C<sub>17</sub>H<sub>26</sub>N<sub>8</sub>O<sub>5</sub>, 1 HCl

**Molecular weight:** 458.9

**pKa values:** 2.8, 4.2, 8.2 and 12.5

### Structure:



### References:

1. PEREZ-GONZALEZ J.A. et al. (1990). Gene.86: 129-134.
2. IZUMI M. et al. (1991). Exp.Cell Res.197: 229-233.
3. ITAYA M. et al. (1990). J.Biochem. 107: 799-801.
4. KIMURA M. et al. (1994). Mol. Gen. Genet. 242: 121-129.
5. KIMURA M. et al. (1994). Biochim.Biophys.Acta.1219:653-659

## RESISTANCE TO BLASTICIDIN S

Three blasticidin resistance genes have been cloned and sequenced: an acetyl transferase gene, *bls* from a blasticidin producer strain<sup>1</sup>, and two deaminase genes, *bsr* gene from *Bacillus cereus*<sup>2,3</sup>, and *BSD* gene from *Aspergillus terreus*<sup>4,5</sup>. Both *bsr* and *BSD* genes are used as dominant selectable markers for gene transfer experiments in mammalian and plant cells. Although Blasticidin S was developed as a selection agent for mammalian cells, the *bsr* and *BSD* resistance genes can also be used in *E. coli*.

## CONDITIONS OF SELECTION

### - *Escherichia coli*

*E. coli* is poorly sensitive to Blasticidin S, but transformants resistant to Blasticidin S can be selected on low salt LB agar medium, pH 8, supplemented with 100 µg/ml Blasticidin S. High pH enhances activity of Blasticidin S. For optimum results, the use of InvivoGen's Fast-Media® Blas is recommended.

### - Mammalian cells

The working concentration of Blasticidin S for mammalian cell lines varies from 1 to 50 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. Suggested working conditions for selection in some mammalian cells are listed below:

Cell line	Species	Tissue	Culture medium	Blasticidin µg/ml
HeLa	Human	Uterus	DMEM	3-10
293	Human	Kidney	DMEM	3-10
B16	Mouse	Melanoma	RPMI	3-10
PC1.0	Hamster	Adenocarcinoma	RPMI	10-30

## METHOD (Selection procedure for mammalian cells)

Blasticidin S is normally used at a concentration of 10 µg/ml. After transfection with a plasmid containing the *bsr* or *BSD* gene, cells are incubated in their regular growth medium containing Blasticidin S to select for stable transfectants.

1- 48 hours post-transfection, pass cells (direct or diluted) in fresh medium containing Blasticidin at the appropriate concentration.

**Note:** Antibiotics work best when cells are actively dividing. If the cells become too dense, the antibiotic efficiency will decrease. It is best to split cells such that they are not more than 25% confluent.

2- Remove and replace antibiotic containing medium every 3-4 days.

3- Evaluate cells for the formation of foci after 7 days of selection. Foci may require an additional week or more to develop depending on the host cell line and transfection/selection efficiency.

4- Transfer and pool 5-10 resistant clones to a 35mm cell culture plate and maintain on selection medium for an additional 7 days. This pooled culture will be expanded for subsequent cytotoxicity assays.

## TECHNICAL SUPPORT

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