

Blasticidin Protocol and Selection Guide

Background

Blasticidin S HCl is routinely used as a selective agent for bacterial and mammalian cells that have been transformed/transfected with plasmids harboring blasticidin resistance genes, namely bsr and BSD. The information below outlines the preparation, use, and storage of blasticidin S HCl.

Preparation and storage

- Prepare stock solution of 10 mg/mL and aliquot into volumes appropriate for individual, one time usage.
- Stock solutions can be stored at 4C for up to 2 weeks (as well as media) and up to 8 weeks at -20C.

Mammalian Cell Culture

Working concentration

The working concentration of blasticidin S HCl for use in mammalian cell gene selection can range anywhere from 2-100µg/mL depending on the cell lines used. Optimal doses typically lie in the range of 2-20µg/mL; however, we recommend performing a kill curve to determine the optimal dose for your cell lines. See below:

- Plate 5×10^4 blasticidin sensitive host cells in 6-12 wells of a 24-well plate using appropriate media* (*without* blasticidin) and incubate overnight.
- Replace media with fresh media containing 6-12 different concentrations (one for each well) of blasticidin S HCl.
- Continue replacing media with fresh blasticidin S HCl containing media every 3-4 days and monitor cells for normal activity.
- After 5-7 days, determine the lowest concentration of blasticidin S HCl containing media that killed 100% of blasticidin sensitive host cells – this is the optimal dose to use for your selection procedure.

Selection of *E. coli*

Working Concentration

The working concentration for blasticidin resistant *E. coli* is usually higher (50-100 µg/mL) than the normal optimal concentrations used in mammalian cell gene selection.

- Plate suspected resistant cells on LB medium* containing 50 µg/mL and incubate overnight.
- The following day, check for individual colonies. If the plate contains confluent growth/lawn, re-plate the cells using 100 µg/mL and incubate overnight.

*Media conditions can inactivate/reduce blasticidin potency, salt levels and pH should not exceed 90mM and 8.0 respectively.

References:

Izumi, M. et. al. "Blasticidin S-resistance Gene (bsr): A Novel Selectable Marker for Mammalian Cells." *Experimental Cell Research* 197.2 (1991): 229-33. *Nih.gov*. PubMed. Web. 20 Sept. 2013.

Kimura, M. et. al. "Blasticidin S Deaminase Gene from *Aspergillus Terreus* (BSD): A New Drug Resistance Gene for Transfection of Mammalian Cells." *Biochimica Et Biophysica Acta*. 1219.3 (1994): 653-59. *Nih.gov*. Web. 20 Sept. 2013.

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<http://cell-lines.toku-e.com>