

## Background

Blasticidin S Hydrochloride (syn: Blasticidin S HCl; TOKU-E item # B001) is a nucleoside antibiotic derived from *Streptomyces griseochromogenes* and is routinely used as a selective agent for bacterial and mammalian cells that have been transformed or transfected with plasmids containing Blasticidin resistance genes, namely *bsr* and *BSD*.

Before stable transfected cell lines can be selected, the optimal Blasticidin S HCl concentration needs to be determined by performing a kill curve titration. The optimal concentration of Blasticidin S HCl suitable for selection of resistant mammalian clones depends on the cell lines, media, growth conditions, and the quality of Blasticidin S HCl, but typically lies between 1 µg/mL - 30 µg/mL. Because of these variables, it is necessary to perform a kill curve for every new cell type and new batch of Blasticidin S HCl.

## Preparation and storage of Blasticidin S HCl solution:

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

## Protocol

1. Seed cells of the parental cell line in a 24-well plate at different densities (50,000 – 100,000 and 200,000 cells/ml) and incubate the cells for 24 hours at 37°C.
2. Remove medium and then add medium with varying concentrations of antibiotic (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 µg/ml) and incubate at 37°C.
3. Refresh the selective medium every 3-4 days and observe the percentage of surviving cells over time (e.g. by EMA vs Hoechst staining or MTT assay).
4. Determine the lowest concentration of antibiotic that kills a large majority of the cells within 14 days. This concentration should be used for selection of a stable transfected cell line.
5. If necessary, repeat the experiment to narrow the antibiotic concentration range.