Blasticidin S HCl Kill Curve Protocol

Background:

Blasticidin S HCl 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 4C for up to 2 weeks (as well as media) and up to 8 weeks at - 20C.

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.