Puromycin DiHCl Kill Curve Protocol

Background:

Puromycin DiHCl is an aminonucleoside antibiotic derived from *Streptomyces alboniger* and is routinely used as a selective agent for mammalian cells that have been transformed or transfected with plasmids containing the puromycin resistance gene, pac.

Before stable transfected cell lines can be selected, the optimal puromycin DiHCl concentration needs to be determined by performing a kill curve titration. The optimal concentration of puromycin DiHCl suitable for selection of resistant mammalian clones depends on the cell lines, media, growth conditions, and the quality of puromycin DiHCl but typically lies between 1 µg/mL - 10 µg/mL. Because of these variables, it is necessary to perform a kill curve for every new cell type and new batch of puromycin DiHCl.

Preparation and storage of puromycin DiHCl solution:

- Puromycin DiHCl is soluble in water at 50 mg/mL yielding a clear, colorless to faint yellow solution. The stock solution may be passed through a 0.22 µm filter and stored in aliquots at –20 °C.

Kill curve/puromycin DiHCl titration 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, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 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, flow cytometry, 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.