

Hygromycin B Kill Curve Protocol

Background:

Hygromycin B is a unique aminoglycoside antibiotic produced by *Streptomyces hygroscopicus* and is routinely used as a selective agent in cell culture and microbiology applications to isolate transfected, hygromycin B resistant cells.

Before stable transfected cell lines can be selected, the optimal hygromycin B concentration needs to be determined by performing a kill curve titration. The optimal working concentration of hygromycin B suitable for selection of resistant mammalian clones depends on the cell lines, media, growth conditions, and the quality of hygromycin B. Because of these variables, it is necessary to perform a kill curve for every new cell type and new batch of hygromycin B.

Preparation and storage of hygromycin B solution:

- Hygromycin B is soluble in water at >50 mg/mL. It is also soluble in methanol or ethanol. Solutions should be sterilized by filtration, not by autoclaving.
- Hygromycin B solutions have been reported to lose activity on freezing. Since solutions are stable refrigerated, freezing should be avoided. Hygromycin B products should be stored as supplied at 2-8 °C. The dry solid is stable for at least five years if stored at 2-8 °C. Hygromycin B solutions are stable as supplied for two years if stored at 2-8 °C.

Kill Curve/Hygromycin B 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, 50, 100, 200, 400, 600, 800, and 1,000 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.