G418 Disulfate Kill Curve Protocol

Background:

G418 disulfate, also known as G418 sulfate, is routinely used to select for successfully transfected mammalian cells that express the neo resistance gene in addition to the gene of interest. The neo gene encodes amino-glycoside 3'-phosphotransferase; an enzyme which confers resistance to G418 disulfate and neomycin.

Before stable transfected cell lines can be selected, the optimal G418 disulfate concentration needs to be determined by performing a kill curve titration. The optimal concentration of G418 disulfate suitable for selection of resistant mammalian clones depends on the cell lines, media, growth conditions, and the quality of G418 disulfate. It is necessary to perform a kill curve for every new cell type and new batch of G418 disulfate.

Preparation and storage of G418 disulfate solution:

- Stock solution - dissolve G418 disulfate in water at a concentration of 50 mg/ml.
- Sterile filter the solution using 0.45ilter.
- Store solution at 2-8°C after use.

Kill curve/G418 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, 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.