TOKU-E G418 Disulfate Kill Curve Protocol

Background

G418 Disulfate (G418 Sulfate; Geneticin)(TOKU-E item # G001) 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 a resistant mammalian clones depends on the cell line, medium, growth condition, and 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:

- Dissolve G418 Disulfate in water at a concentration of 50 mg/ml to prepare stock solution.
- Sterile filter the solution using a 0.45 µm filter.
- Store stock solution at 2-8°C.

Note: We also offer G418 Disulfate Solution (50 mg/ml in Water)(TOKU-E Item # G020)

Kill curve/G418 titration:

1. Seed cells of the parental cell line in a 24-well plate at different densities (50,000 – 100,000 and 200,000 cells/well 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.

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