PROTOCOL

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INSPIRED CELL SOLUTIONS

Dose response curve for antibiotic selection of mammalian cells (kill curve)

Mammalian cell sensitivity to antibiotics varies from one cell type to another. In order to generate a stable cell line expressing a transgene or shRNA of interest, it is important to determine the minimum concentration of antibiotic required to kill non-transfected (plasmid DNA) or non-transduced (e.g., lentiviral particles) cells. Antibiotic selection typically begins 24-48 hours after transfection or transduction. The following protocol provides general guidelines for determining the concentration of antibiotic needed to select mammalian cells.

Materials required

- Multi-well tissue culture plates or tissue culture dishes
- Antibiotic specific to the resistance gene encoded by plasmid DNA or lentiviral vector. Examples used in this protocol
  - *Blasticidin S* (Fisher Scientific, Cat #BP2647-25; InvivoGen, Cat #ant-bl-1)
  - Puromycin (GE Life Sciences HyClone, Cat # SV30075.01; InvivoGen Cat # ant-pr-1 Fisher Scientific, Cat #BP2956-100)
- Growth medium: the cell culture medium (including serum or supplements) recommended for maintenance and passaging of the cells of interest
- Selection medium: growth medium supplemented with the appropriate concentration of the antibiotic for cell selection

Protocol for antibiotic kill curve in adherent cells

Day 1

Using the same cell type and relative cell densities to be used in subsequent transfection or transduction procedures, plate cells and culture overnight under appropriate conditions (e.g., 37 °C with 5% CO_2).

Note: Seed enough cells to be 25–50% confluent on the day antibiotic selection will be initiated. After transfection or transduction (24–48 hours), cells typically will need to be passaged.

Day 2

Replace complete Growth Medium with Selection Medium supplemented with a range of antibiotic concentrations. Include untreated control cells with Growth Medium only (no selective antibiotic added).
Note: Typical antibiotic working concentration range for mammalian cells is 0.5–10 µg/mL for puromycin and 1-20 µg/mL for blasticidin (Figure 1).

Days 4–15

1. Monitor the cells daily using a microscope and observe the percentage of surviving cells. Optimum effectiveness should be reached in 2-15 days for most cell lines depending on the antibiotic used:
   - Puromycin: 2–7 days
   - Blasticidin: 3–15 days
2. Approximately every 2–3 days replace medium with freshly prepared Selection Medium containing the range of antibiotic concentrations being tested.
3. The minimum antibiotic concentration to use is the lowest concentration that kills 100% of untreated control cells in 2–15 days from the start of antibiotic selection.

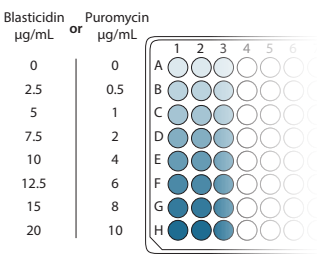

Figure 1. Example of plate layout for 96-well format to assess optimal concentration of antibiotic for selection, using puromycin or blasticidin as examples, of cells expressing a transgene or shRNA of interest.

If you have any questions

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