

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% CO₂).

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 controls cells in 2-15 days from the start of antibiotic selection.

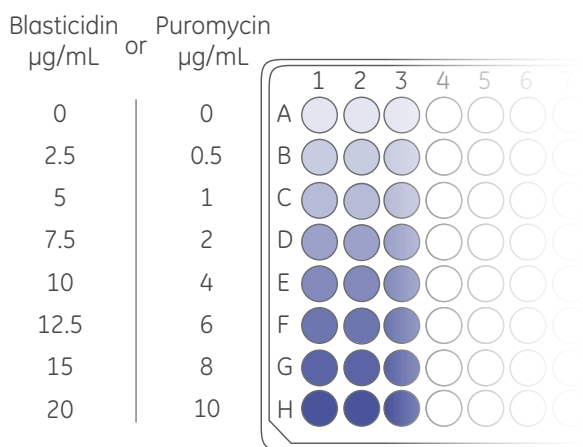


Figure 1. Example of plate layout for 96-well format to assess optimal concentration of antibiotic for selection, using puromycin or blasticidin as examples, of cells expressing a transgene or shRNA of interest.