DRUG-SELECTION KILLING CURVE

For stable cell line generation, it is important that stably-transfected/transduced cells are selected by the addition of antibiotic drugs to the culture medium, if the expression system carries a drug resistance gene. Generation of a selection-drug killing curve will help determine the minimum amount of the selection-drug required to kill non-transfected/transduced cells.

Protocol
To determine a drug killing curve:

1. Plate cells into a 6-well plate with the density around 2 x 10^5 cell per well.
2. Prepare medium containing a range of antibiotics (ie. 0-15µg/ml puromycin; 0.1-1.5mg/ml G418). On day 2 replace the growth media with media containing the dilutions of the antibiotic into the wells.
3. Feed cells every 2-3 days with freshly prepared selection media.
4. Monitor the cells daily and observe for cell death. Optimum effectiveness should be reached in 1-4 days with puromycin, and 7-14 days with G418.
5. Choose the concentration which shows complete cell death as the minimum antibiotic concentration to use for selection.

Drug selection:

After 48 hours post-transfection/viral transduction, replace the growth media with fresh growth media containing the appropriate antibiotic concentration.

For laboratory research only. Not for clinical applications. For technical questions, please email us at technical@abmgood.com

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