Protocol

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Developing a Titration Kill Curve (Adherent Cells)

Utilizing G418, Hygromycin B or Puromycin

Introduction

A Kill Curve is a dose response experiment which tests the amount of antibiotic resistance in a cell line using incremental doses that determine the minimum antibiotic concentration required to kill all of the cells. Each mammalian cell line has a different sensitivity. Before experimentation, you should determine the optimal concentration of your antibiotic by developing the Kill Curve titration for your cells as detailed below.

Materials

- DMEM (Dulbecco's Modified Eagle Medium)
- 10% FBS (Fetal Bovine Serum)
- G418 [G418 Sulfate, GoldBio Catalog # G-418, (MW = 692.71 g/mol)]
- Hygromycin B [Hygromycin B, GoldBio Catalog # H-270, (MW = 563.5 g/mol)]
- Puromycin [Puromycin Dihydrochloride, GoldBio Catalog # P-600, (MW = 544.43 g/mol)]

Method

1. Generate a cell suspension from adherent cells at 1:5 in 10 ml DMEM + 10% FBS media. Cells may be harvesting either by cell scraping the dish or by Trypsinization.

2. Transfer 0.5 mL cell suspension into 24-well plate containing 500 µl of (media + antibiotic).
    a. Antibiotics to use: G418, Hygromycin B or Puromycin.

3. Examine viability every 2 days.

4. Culture for 14 days. Replace the media containing antibiotic every 3 days.

5. Use the lowest concentration of your antibiotic that begins to give massive cell death in 3 days and kills all the cells within two weeks. A general starting point is usually 400 µg/ml G418 for HeLa cells and 200 µg/ml hygromycin for CHO cells. In mammalian cells the optimal level of puromycin is typically around 1 µg/ml. HeLa cells are often selectable with 500 µg/ml G418, 500 µg/ml hygromycin, or 2.5 µg/ml puromycin, and SHSY-5Y cells are often selectable with 600 µg/ml G418 or 200 µg/ml hygromycin.

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Calculations

Volume of Stock Solution Added (µl)		Final Concentration (µg/ml)	Volume of Stock Solution Added (µl)	Final Concentration (µg/ml)
G418 (200 mg/ml)	Hygromycin B (50 mg/ml)		Puromycin (2.5 mg/ml)	
0	0	0	0	0
0.25	1	50	0.2	0.5
0.5	2	100	0.4	1.0
0.75	3	150	0.6	1.5
1.0	4	200	0.8	2.0
1.5	6	300	1.0	2.5
2.0	8	400	1.2	3.0
2.5	10	500	1.6	4.0
3.0	12	600	1.8	4.5
3.5	14	700	2.0	5.0
4.0	16	800	2.4	6.0
5.0	20	1 mg/ml	3.0	7.5