Developing a Titration Kill Curve (G418, Hygromycin B and Puromycin)

Sean Seaver

Abstract

Developing a Titration Kill Curve (G418, Hygromycin B and Puromycin)

Citation: Sean Seaver Developing a Titration Kill Curve (G418, Hygromycin B and Puromycin). **protocols.io** dx.doi.org/10.17504/protocols.io.ci9uh5
Published: 03 Nov 2014

Before start

Each mammalian cell line has a different sensitivity. Before experimentation, you should determine the optimal concentration of your antibiotic by developing the Titration Kill Curve for your cells.

Materials

G418 Disulfate LGB-418 by P212121

Hygromycin B GB-T005 by P212121

Puromycin Dihydrochloride RP-P33020 by P212121

Protocol

Step 1.
Split non-transduced, confluent cells 1:5 in 10 ml DMEM + 10% FBS media.

DURATION
00:05:00

Step 2.
Transfer 0.5 mL cell suspension into 24-well plate containing 500 µl of (media + drug).

- G418 Sulfate
- Hygromycin B
- Puromycin Dihydrochloride

DURATION
00:08:00

Step 3.
Examine viability every 2 days.

DURATION
48:00:00

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

DURATION
12:00:00

Step 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 mg/ml G418 for HeLa cells and 200 mg/ml hygromycin for CHO cells.

In mammalian cells the optimal level of puromycin is typically around 1 mg/ml.

HeLa cells are often selectable with 500 mg/ml G418, 500 mg/ml hygromycin, or 2.5 mg/ml puromycin, and SHSY-5Y cells are often selectable with 600 mg/ml G418 or 200mg/ml hygromycin.

REAGENTS
Hygromycin B GB-T005 by P212121