Aug 09, 2018

# G418 Kill curve protocol

DOI
dx.doi.org/10.17504/protocols.io.sh4eb8w

Fatma Gomaa[1], Zhuhong Li[2], Roberto Docampo[2], Peter Girguis[1], Virginia Edgcomb[3]
[1]Harvard University; [2]University of Georgia; [3]Woods Hole Oceanographic Institution

Protist Research to Opti...

Fatma Gomaa
Harvard University



**DOI: dx.doi.org/10.17504/protocols.io.sh4eb8w**

**Protocol Citation:** Fatma Gomaa, Zhuhong Li, Roberto Docampo, Peter Girguis, Virginia Edgcomb 2018. G418 Kill curve protocol . **protocols.io https://dx.doi.org/10.17504/protocols.io.sh4eb8w**



**Protocol status:** Working
**We use this protocol and it's working**

**Created:** August 09, 2018

**Last Modified:** August 09, 2018

**Protocol Integer ID:** 14620

## Abstract

***Bodo saltans* kill curve protocol using G418 (Gentamicin)**

- Gentamycin exhibits toxicity toward both eukaryotic and prokaryotic cells by disrupting ribosome function, thereby blocking the elongation step in protein synthesis.
- G418 is most commonly used as a selection agent for eukaryotic cells genetically engineered to express a neomycin resistance gene (NeoR), which is encoded by either transposon Tn601 (903) or Tn5.
- The resistant cells grow in medium containing G418, and may be used to establish stably transfected cell lines as all the non-resistant cells die due to G418 toxicity, typically within 6 – 14 days.
- To establish kill curve assay for *saltans* various concentrations of G418 were tested. The concentration range from 1 to 50 µg/mL for selection of eukaryotic, and then higher concentrations can be used for maintaining stable transfected cell line.

**Kill Curve Assay**

1. Harvest the *B. saltans* cells from a culture that is at peak density (1 – 3.0 x $10^5$) by centrifugation at 800 x g for 5 min., discard the supernatant (medium).

1. Replace the growth medium with fresh medium containing 0 – 50 µg/mL. For each concentration, test in triplicate.

1. Using 6 wells plates, replace the medium in the wells every 3 – 4 days using fresh medium with the appropriate G418 concentration. Perform a daily visual inspection for evidence of toxicity, also cell count using hemocytometer.

1. Note that the optimal dose of G418 for selection is the lowest one for which all cells have died after one week. A low dose is the concentration which has minimal effects on cells after 2 weeks of antibiotic selection. Conversely, a high dose is a concentration which is highly toxic to cells within 2-3 days of starting antibiotic selection.

5- The results indicate that a G418 concentration of 2 µg/mL kill the entire *B. saltans* population in 12 days which is considered enough time for selecting the resistant cells after transfection.

**G418 Sensitivity for *B. saltans***

| | G418 (µg/ml) | Cells survival (days) |
|---|---|---|
| | 50 | 2 |
| | 20 | 2 |
| | 15 | 3 |
| | 10 | 4 |
| | 5 | 6 |
| | 3 | 8 |

| | |
|---|---|
| 2 | 12 |
| 1 | >16 |
| 0 | >16 |

| |
|---|
| G418 (µg/ml) |
| Cell survival (days) |

**Cell Selection**

1. In all of our plasmids constructs we included the *NeoR* gene (Neomycine resistant gene) to select our transfected cells.
2. Twenty-four hours after electroporation, the growth medium of transfected cells were supplemented with G418 antibiotic Solution (2 µg/mL) based on the kill curve results (above).

1. 3. Replace the G418-containing medium every 3 – 5 days and examine cells for visual toxicity. Most non-transfected (non-resistant) cells will die within 10- 12 days, leaving the transfected cells to expand.
2. Once cells grow to high confluence, they may be maintained, or frozen as a polyclonal line or plated by limited dilution to select for single clones.

## Attachments

DOCX

Kill curve_B_saltans...