Protocol for drug sensitivity assay with S. robusta

Dan Needleman

Abstract

Citation: Dan Needleman Protocol for drug sensitivity assay with S. robusta. **protocols.io** dx.doi.org/10.17504/protocols.io.g9ebz3e
Published: 23 Feb 2017

Protocol

Developed by Aaron Turkewitz and Lev Tsypin

Step 1.

1) Measure density of floating cells in culture (D6 strain):

a. Grow 100 mL volume of cells in a 175 cm^2 flask for three to four days, until there is a substantial number of healthy, floating cells

b. Stand flask vertically and take a volume of the floating culture (so as to not disturb the adherent cells) to measure cell density

c. Count floating cells (you may need to concentrate them, prior to counting, e.g., in a haemocytometer)

2) Distribute cells on a petri dish:

a. The target concentration for distributing cells is 400-500 floating cells/mL, so dilute as necessary. The volume needed for a plate is 2 mL, including the volume of any added drugs.

b. Transfer 2 mL, containing the desired number of cells, to an Eppendorf tube.

i. Centrifuge using a personal mini-centrifuge for two minutes. At this speed (which on our model is 6000 rpm, 1400 x g), many cells remain in the supernatant. Those are the cells to distribute in drops. Do not disturb cells in the pellet.

Developed by Aaron Turkewitz and Lev Tsypin

Step 2.