NYU School of Medicine
Department of Microbiology

Ana Rodriguez, Ph.D.
Associate Professor
Ana.Rodriguez@nyumc.org

Screening of drugs against Plasmodium falciparum (3D7 strain) *in vitro*

The assay is performed in 96 well sterile plates for 96 hours. Each compound should be tested in duplicate and controls in triplicate.

Reagents

Plasmodium falciparum 3D7 erythrocytic asexual culture, at 5% hematocrit maintained in the atmospheric conditions of 1% oxygen, 5% carbon dioxide and 94% nitrogen
Complete media (RPMI 1640, 25 mM HEPES, 10 ug/ml gentamycin, 0.5 mM hypoxanthine, pH 6.75), supplemented with 25 mM sodium bicarbonate and 0.5% Albumax II
SYBR Green I nucleic acid staining dye (Molecular Probes), 10000X stock, stored at -20°C
Lysis Buffer (20 mM Tris at pH 7.5, 5 mM EDTA, 0.008% saponin, 0.08% Triton X-100)

Culture

Maintain cultures by changing medium daily and keeping parasitemia below 6%. When performed, parasite cultures are synchronized using MACS cell separation column (Miltenyi Biotec). To determine parasitemia, the number of parasitized erythrocytes from 500 cells in a Giemsa stained blood smear are counted.

Controls

Medium, Red Blood Cells, Parasite, Parasite + 100 µM Chloroquine

Drug Treatment:

1. Add 100 µL of warm (37°C) complete media containing drug to each well of a 96 multi-well clear, sterile plate.
2. Add 100 µL of *Plasmodium falciparum* 3D7 culture to each well containing drug at 0.25% ring-stage parasitemia (synchronous) and 10% hematocrit (final hematocrit will be 5%).
3. Maintain treated cultures under the atmospheric conditions of 1% oxygen, 5% carbon dioxide and 94% nitrogen for 96 hours.

4. After 96 hours of growth*, freeze at -80°C overnight. **Note: Growth can be examined by pipetting 5 µL from desired wells onto slides and creating blood smears. Do not mix wells prior to pipetting and pipette from bottom of well where blood has settled.*
5. After freezing, thaw at 37°C for 4 hours.
6. Transfer 100 µL, pipetting wells up and down to mix, to a black, sterile 96 well plate.
7. Create working solution of 0.2 µL of SYBR Green per 1 mL of lysis buffer, and add 100 µL of this solution to each well, pipetting up and down to mix/lyse erythrocytes. **Note: mixture should be a translucent red color.*
8. Incubate at room temperature, protected from light, on a shaker for 1 hour.
9. Measure the fluorescence using excitation and emission wavelengths of 485 and 530 nm, respectively.

Reference: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC400546/pdf/0851-03.pdf

November 1, 2016