P. falciparum Asexual Blood Stage (ABS) assay

General assay principle:

This assay is adapted from Plouffe et al 2008. In short, we are using the *Plasmodium falciparum* (DD2 strain) blood stage growth as indirectly measured through DNA amount with SYBR green. Blood stage parasite cultures are treated with compound in 1536 well plates and incubated for 72 hours. Whole well fluorescence is then measured after addition of SYBR green and is used to evaluate parasite growth.

This assay allows us to identify compounds with activity against the symptomatic parasite blood stages.

Parasite culture.

The *P. falciparum* Dd2 strain is cultured in Complete Medium (RPMI 1640 Medium with L-Glutamine, 0.05 mg/ml Gentamicin, 0.014 mg/ml Hypoxantine, 38.4 mM HEPES, 0.2% Sodium bicarbonate, 0.2% Glucose, 3.4 mM NaOH, 4.3% Human Serum, 0.2% Albumax) supplemented with 5% hematocrit until parasitemia reaches 3-8%. Parasitemia is estimated by blood smear Giemsa stain and visual inspection under the microscopic.

Screening plate preparation.

Centrifuge for parasite culture for 5 min at 800x g at RT (low brake) and remove supernatant. Prepare a parasite suspension with 0.3% parasitemia and 4% hematocrit in screening medium (SM). Gas the suspension with a gas mixture of 1% oxygen, 3% carbon dioxide, 96% nitrogen and store at 37°C for until ready to use.

Prespot 50 nl of 1 mM compound in DMSO into 1536 well plates (final drug conc. of 12.5 µM, final DMSO conc. of 0.625%) with the acoustic dispenser ATS. Controls are Artemisinin, Chloroquine, and DMSO.

Dispense 8 µl of SM into 1,536-well, black, clear-bottom plates (Greiner) using the MultiFloTM Microplate dispenser (BioTek) (final parasitemia of 0.3% and 2.5% hematocrit in SM).

Incubate plates at 37°C for 72 h with water soaked tissue in a closed ziploc bag gassed with 1% oxygen, 3% carbon dioxide, 96% nitrogen.

Data Acquisition.

Add 2 µl of detection reagent mixture (10x SYBR Green I (Invitrogen) in Lysis buffer (20 mM Tris/HCl, 5 mM EDTA, 0.16% Saponin wt/vol, 1.6% Triton X vol/vol) and incubate the plates at RT for 24 h in the dark.

Read assay plates from the bottom by using the 2104 EnVision® Multilabel Reader (PerkinElmer) (485 nm excitation, 530 nm emission).

Literature reference and notes:

Plouffe D et al (2008) “In silico activity profiling reveals the mechanism of action of antimalarials discovered in a high-throughput screen.” Proc Natl Acad Sci U S A. 2008 Jul 1;105(26):9059-64