

Screening of drugs against *Trypanosoma T. brucei brucei* (427 strain) *in vitro*

The assay is performed in 96 well white sterile plates for 48 hours. Each compound and controls are tested in triplicates.

Reagents:

HMI-9 medium - Iscove's modification of DMEM (IMDM; Cell Gro) supplemented with 10% FBS, 10%, Serum plus (SAFC), 0.05 mM Bathocuproinesulfonate, 1.5 mM L-cysteine, 1 mM hypoxanthine, 0.2 mM β -mercaptoethanol, 0.16 mM thymidine 1mM pyruvate (stock: 100mM).
Alamar Blue (Sigma)

Controls:

Medium alone, parasites alone, parasites + 100uM of Ionomycin (or 100uM Suramin)

Protocol:

- Thaw out compounds
- Spin parasites for 10 min at 900g (2 acceleration, 0 break).
- In the meanwhile, add 100uL of HMI-9 medium per well in the 96 well white plate
- Vortex compounds
- Add 2uL of each compound + 98uL of fresh medium in the first row, pipette up and down to mix and then transfer 100uL to the next row in order to make serial dilutions
- After centrifugation, discard medium by aspiration carefully
- Resuspend parasites in warm medium and count them in Newbauer chamber
- Dilute parasites at 5×10^4 cells/ml (5×10^3 cells/well) and plate 100uL per well with multichannel pipette
- After 48 hours, add 20uL of Alamar blue per well
- Incubate at 37°C for 4 hours
- Read fluorescence at excitation 530nm and 590nm emission wavelength