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Screening of drugs against Trypanosoma cruzi (Tulahuen strain) *in vitro*

Growth inhibition assay in 96 well plates:

- Final volume per well for cells+parasites+compound is 200 µl.
- Final volume after adding substrate is 250 µl.
- Compounds are tested in duplicate or triplicate.
- The assay is performed in DMEM **without Phenol red +** 2% FBS and 1% PSG to avoid interference of phenol red with the 590 nm absorbance reading.
- **Controls:**
    1. cells + parasites
    2. cells without parasites
    3. cells+parasites+Amphotericin B 4 µM (2.96 µL of 270 µM stock/well)
    4. medium only

Protocol:

- harvest parasites (T. cruzi-Tulahuen expressing β-galactosidase) in 50 ml tubes and spin 7 min at 2500 rpm
- Rinse parasites 2x with DMEM **without Phenol red + 2% FBS and 1% PSG.**
- spin again at 2500 rpm for 7 min. take tubes cautiously out of the centrifuge, place them on a rack in the incubator to let trypomastigotes swim out of the pellet, for 3-5 hours.
- In the meantime, plate 100µL (**50.000 NIH 3t3 cells**) per well with multichannel pipette.
- Put back in incubator for 3 hours to allow cells to attach.
- Thaw compounds and add them at desired concentration
- Add 100µL of parasite (**50.000 trypomastigotes)** in each well. Incubate for 4 days
- Add 50 µL of substrate solution (500 µM CPRG in PBS+0.5% NP404) per well of 96 well plate with multipipette.
- Incubate for **4 hours**
- Read absorbance at 590-595 nm

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