**Fixation of *T. brucei* (PCF) for GFP fluorescence and DAPI staining**

**Updated January 2019**

1. Count cells. Spin down 1E7 cells per sample at 800 rcf for 5 min.
2. Wash 1X with 1 vol PBS.
3. Resuspend in 1 ml PBS. Outline areas on charged slide with lipid pen.
4. Add 200 µl/area of washed cells to slide. Allow cells to adhere for 10 min in humid chamber.
5. Tilt excess liquid onto paper towel, then wash 1X briefly and 1X 5 min in PBS in Coplin jars. During 5 min wash take 4% paraformaldehyde in PBS out of fridge.
6. Return slides to humid chamber. Add 200 µl per area of (cold) 4% paraformaldehyde. Incubate for 15 min.
7. Remove excess liquid. Wash 1X briefly and 2X 5 min in PBS (Coplin jars).
8. Prepare DAPI staining solution by diluting 1 µl of 2 mg/ml stock in 1 ml PBS to give 2 µg/ml final.
9. Return washed slides to humid chamber. Add 200 µl per area of DAPI solution. Incubate for 5 min.
10. Remove excess liquid. Wash 1X briefly and 1X 5 min in PBS (Coplin jars).
11. Mount with 10 µl per area of Vectashield. Seal edges of coverslip with nail polish.