**FACS analysis**

**Fixation:**

Need 2 x 10⁷ trypanosomes for each analysis.

Harvest cells by centrifugation at 1,500 rpm, 10 min at 4ºC

Wash twice with cold PBS (PF) or TDB (BF) +2 mM EDTA.

Resuspend cells in 200µl PBS (PF) or TDB (BF) +2 mM EDTA in a 15 ml conical tube

Fix by adding 2 ml of ice-cold 70% EtOH dropwise while vortexing on high speed

Store at 4ºC for at least overnight: cells are stable for weeks

**Staining:**

Spin cells for 10 min at 1,500 rpm at 4ºC

Resuspend pellet in 0.5 ml staining solution: per ml: 950µl PBS/2 mM EDTA

20µl RNAseA 10 mg/ml

50µl Propidium Iodide (1 mg/ml)

Incubate at 37ºC for 30 min

Proceed with FACS analysis