IF of living cells (in suspension) for microscopy/FACS

Starting material > 10^6 and preferably 10^7 cells.

For microscopy, BSG seems to be a good buffer. For FACS, use HMI-9. BSG is not good. It contains particulate salts in suspension that lead to a very high noise that we cannot distinguish from a tryp (probably due to high Ca concentration: see alternative BBS-based buffers in the 'protocol buffers etc general-purpose' file).

Owing to the rapid endocytosis of VSG-Ig complexes, all the procedures MUST be performed at 0–4°C: all incubations should be done on ice. Use a refrigerated centrifuge at 0°C, not a non-refrigerated centrifuge in a cold room.

Handling was performed under the hood, so that parasites do not get contaminated. Use glass conical tubes. Sera should be pre-centrifuged to remove any aggregates.

IF for FACS or micrcoscopy

1. Spin 10^6 cells at 0°C in a conical glass tube.
2. Resuspend to $1x10^7$/ml in BSG containing 1% BSA at 0°C.
3. To 100 µl of cells add 100 µl of anti-VSG serum diluted 1:200, or as appropriate. Higher concentrations are needed for live tryps because only one epitope is probably visible on VSG in situ, and antibodies are rapidly endocytosed.
4. Incubate cells for 30 min on ice.
5. Add 5–10 ml of BSG/BSA, gently shake tube and spin.
6. Resuspend cells in 100 µl of BSG/BSA.
7. Add 100 µl of 1:100 fluorescent conjugated anti-rabbit diluted in BSG/BSA.
8. Incubate cells for 30 min at 4°C.
9. Add 10 ml of BSG/BSA, gently shake tube and spin.
10. Resuspend in 1 ml of BSG/BSA.
11. Perform FACS immediately (cells ALWAYS on ice)
12. If you want to make sure that cells are still viable, place 100 µl back into culture (in 5 ml HMI9). They should reach ~$2x10^6$ cells/ml after 3 days.
13. For microscopy, aliquot 10 µl of living stained cells onto a clean slide and apply a coverslip. This has to be done very quickly (close to the microscope, unless it is in a cold room or has a cooled stage and objective) because once cells are at RT, they will quickly internalize the antibody (but they can be fixed at this stage, if desired).