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Sample Preparation Guide

Core Facility Flow Cytometry Berg

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1. **Protocols:** In general, any protocols used for flow cytometry will work with the Image Stream. Use 1,5ml polypropylene tubes to process samples.
2. **Cells:** cells/particles should be less than 45 um in diameter. If cells tend to aggregate use a **70um cell strainer** and/or an anti-clumping buffer (2mM EDTA)
3. **Choice of Fluorochromes**: Choose 488, 405, 651, 642 nm excitable fluorochromes
4. **Brightness of stain and stain balancing:** use the brightest fluorochrome for the antigen with the smallest copy number. To increase data quality, reagents that are excited with the same laser should be titrated such that brightness levels of all probes are balanced to within a log of each other. Probe balancing avoids saturation of bright stains when combined with dim stains in the same sample.
5. **Controls:** For spectral compensation, it is important to have unlabeled cells and cells labeled with a single-color positive control for each fluorochrome used.
6. **Final sample concentration and volume:** Ideally 20Mio/ml in 50ul in a 1,5ml tube. Lower concentration increase data collection time.
7. **Number of samples:** Please limit your sample to the following:
    1. Positive biological control
    2. Negative biological control
    3. Unlabeled control
    4. Single color control(s)
    5. Experimental samples
8. **Documentation:** A sample list with detailed description of the sample (Data Acquisition Form). If available, Microscope images and/or flow cytometry data.

9. **It takes about 6 minutes to process a single sample and longer if the sample is below the recommended concentration or the target population is very small. Each 10.000 cell image file will occupy about 200MB. (appr. 500MB including analyzed files).To analyze and report data, we allocate at least one full day.**