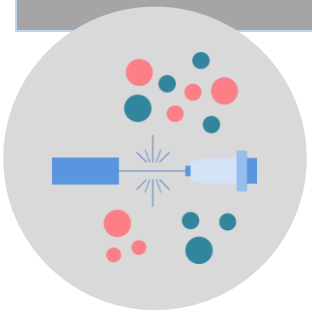

Fluorescence-Activated Cell Sorting Flow Cytometry

www.davids-bio.com (Custom Antibodies)
www.davids-science.de (Lab Material)

- 1 - Introduction

Fluorescence-Activated Cell Sorting (FACS) is a technique used in modern cell biology. It enables researchers to analyze and isolate specific cell populations based on their unique fluorescent characteristics. Cell sorting is an extension of traditional flow cytometry, incorporating the ability to physically sort cells in real-time while simultaneously collecting valuable data on cellular properties.

Flow cytometry is a method that characterizes individual cells within a heterogeneous population by measuring their fluorescence and light-scattering properties. By employing fluorescent-labeled antibodies, researchers can target specific cell surface markers or intracellular proteins. When excited by laser, these fluorochromes emit light of different wavelengths, allowing for simultaneous multi-parameter analysis of the cells. They can be classified into distinct populations based on their fluorescence profiles, and then physically separated into microcentrifuge tubes. Scientists can obtain highly purified cell populations for downstream applications, including gene expression analysis and cell culture.

Material		
Cell Suspension		
Isotype Controls	Normal IgG (use antibodies with the same species like the primary antibody)	www.davids-science.de
FACS Buffer	1x PBS 0.02% NaN_3 1% BSA *(Alternatively: 2% FBS)*	Without Ca_{2+} and Mg_{2+}
Staining Solution	Propidium-Iodide *Alternatively: 7-aminoactinomycin D*	
Fixation Buffer	1% Paraformaldehyde	
Primary Antibody	FITC-conjugated antibodies	
Secondary Antibody		www.davids-science.de

- 2 - Method

Cell Preparation

- Collect and wash the cells with 1 x PBS
- Centrifuge the cells at 350 x g for 5 minutes
- Discard the supernatant and resuspend the cell pellet in FACS Buffer
- Aliquot the cells in reaction tubes to a concentration of 2-5 x 10E6 cells/ml

Cell Staining

- Add 1 µg/ml FITC-labeled antibodies to the cell suspension
  Prepare isotype controls for each antibody used
- Incubate the cells in the dark at 4°C for 30 minutes
- Add 1 µg/ml propidium iodide to the mixture during the last 5 minutes of incubation
- Centrifuge the stained cells at 500 x g for 5 minutes
- Discard the supernatant and resuspend the cell pellet in 1 ml of ice cold FACS Buffer

Washing

- Centrifuge the cell suspension at 500 x g for 5 minutes and discard the supernatant
- Resuspend the cell pellet in 200 µl of 1% paraformaldehyde
- Incubate the cells for 15 minutes at room temperature
- Centrifuge the stained cells at 500 x g for 5 minutes and discard the supernatant
- Resuspend the cells in 1 ml of ice cold FACS Buffer
- Filter the cell suspension through a 60 µm cell strainer

Flow Cytometer

- Turn on the flow cytometer and allow it to warm up
- Calibrate the machine using calibration beads
- Set appropriate parameters, like forward scatter, side scatter and fluorescence detectors
- Perform a sample acquisition to establish the baseline data

Cell Sorting

- Set up appropriate sorting gates based on forward and side scatter properties
- Sort the desired cell population into sterile centrifuge tubes
- After cell sorting, analyze a portion of the sorted cells via flow cytometry again, to confirm the purity and viability of the sorted populations
- Centrifuge the cells at 500 x g for 5 minutes and discard the supernatant
- Resuspend the cell pellet in an appropriate cell culture media

- 3 - Trouble Shooting

Reason	Solution
Low Signal	Optimize antibody concentration and incubation time for cell staining. Check the viability of your fluorophores and avoid overexposure to light during sample preparation. Use appropriate filters and gating to exclude cell clumps and debris.

Reason	Solution
High Background	Use appropriate isotype controls and fluorescence control to identify and subtract background signal. Optimize the blocking step with the blocking agent to minimize nonspecific binding. Consider using samples from knockout or negative controls to assess autofluorescence.
Overlapping Populations	Use different antibody concentrations to optimize signal intensity ranging from 0.1 to 10 µg/ml. Choose fluorophores with minimal spectral overlap. Use additional markers and dyes to better distinguish cell populations.
Inconsistent Results	Maintain low temperatures ranging from 2 - 8°C. Standardize sample preparation procedures and conditions. Establish and adhere to a consistent gating strategy. Run calibration beads to ensure instrument stability. Regularly perform instrument quality control and maintenance.
Cell Aggregates	Optimize cell dissociation protocols to obtain single cell suspensions. Use proper cell strainers and filters to remove clumps and debris. Adjust cell concentration to minimize cell density leading to cell doublets.
Cell Viability	Avoid using sodium azide to FACS Buffer to maintain cell function. Ensure cell viability of >90%. Pre-coat tubes and filters with blocking agents to prevent cell adherence.
Viability Staining	Consider using 7-aminoactinomycin D (7-AAD) instead of propidium iodide (PI) for GC-rich regions of double-stranded DNA.