Flow Cytometry Protocol: An Overview

Flow cytometry is a laser-based technology used to analyze the characteristics of single cells in suspension. This guide outlines the essential steps from initial sample harvesting to final data acquisition, emphasizing the importance of cell viability and blocking to ensure high-quality, low-background results.

Phase 1: Sample Preparation & Quality Control

Create a Single-Cell Suspension

Prepare cells from blood, culture, or tissue using mechanical dissociation or enzymatic digestion.

Adherent Cells

Detach using 10mM EDTA or Accutase Medium

Lymphoid Tissue

Mechanical Disruption using a syringe plunger or glass slides

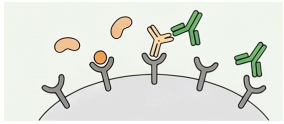
Non-Lymphoid Tissue

Mince into 2-4 mm pieces, followed by enzymatic digestion

Verify Cell Viability

Ensure viability is >90% using dyes like DAPI or 7-AAD to exclude debris.

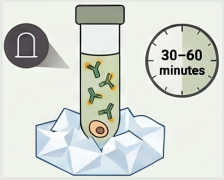
Block Non-Specific Binding



Incubate with 10% scrum to prevent antibodies from binding to Fc receptors.

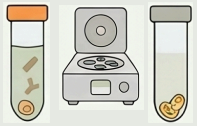
Phase 2: Immunostaining & Data Acquisition

Antibody Incubation



Add fluorochrome-labeled antibodies and incubate in the dark on ice for 30-60 minutes.

Wash & Resuspend



Centrifuge and wash three times with FACS uffer to remove unbound antibodies.

Final Analysis & Storage

Analyze samples immediately or fix with 1-4% paraformaldehyde for later processing.

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