STANDARD OPERATING PROCEDURE		
Document No.	SOP-QC-0147	**Effective Date**
Version	2.0	**Page 1 of 3**
Title	Determination of Cell Viability by 7-AAD Exclusion Using Flow Cytometry	
Department	Quality Control / Analytical Development	

SAMPLE DOCUMENT — Prepared by Kalif Shear as a writing demonstration. Uses only publicly established, generic methodology. Contains no proprietary or employer-confidential information. Document number and dates are illustrative.

1. Purpose

This procedure describes the method for determining the viability of a single-cell suspension by 7-aminoactinomycin D (7-AAD) dye exclusion using flow cytometry. Viability is expressed as the percentage of cells that exclude 7-AAD relative to the total gated cell population.

2. Scope

This SOP applies to viability testing of nucleated cell suspensions performed within the Quality Control and Analytical Development laboratories. It applies to in-process, release, and stability samples where viability is a defined test attribute. It does not apply to methods requiring fixation or membrane permeabilization.

3. Responsibilities

Analyst: Performs the procedure as written, records results contemporaneously, and reports any deviation to the supervisor.

Supervisor / Reviewer: Reviews data for completeness and compliance with acceptance criteria and approves the result.

Quality Assurance: Maintains the controlled version of this document and oversees deviation handling.

4. Materials, Reagents & Equipment

Item	Specification / Example
7-AAD viability dye	Ready-to-use, protected from light, stored 2–8 °C
Staining buffer	PBS with 1–2% FBS or BSA, 0.2 µm filtered
Flow cytometer	Instrument qualified and within calibration; blue (488 nm) laser, ~670 nm detector
Consumables	12 x 75 mm tubes or equivalent; calibrated pipettes

5. Safety

7-AAD is a nucleic-acid intercalating dye and a potential mutagen; handle with gloves and avoid skin contact. Handle all cell material as potentially biohazardous per site biosafety requirements. Dispose of stained material and sharps in designated waste streams.

6. Procedure

6.1 Sample preparation. Bring the cell suspension to a single-cell state and determine the approximate concentration. Adjust to a working concentration suitable for the cytometer (typically 0.5–1.0 x 10^6 cells/mL) using staining buffer.

6.2 Aliquot. Transfer a defined volume of the suspension (e.g., 100 µL) into a labeled tube. Prepare an unstained control from the same suspension.

6.3 Stain. Add the validated volume of 7-AAD to the sample tube. Do not add dye to the unstained control. Mix gently.

6.4 Incubate. Incubate protected from light at the defined temperature and time (typically 10–15 minutes at 2–8 °C or room temperature, per the qualified method).

6.5 Acquire. Acquire a minimum defined number of events (e.g., ≥ 10,000 gated cells) on the qualified cytometer. Use the unstained control to set the negative boundary.

6.6 Gate and analyze. On a plot of the 7-AAD detector, gate the viable (7-AAD-negative) population against the unstained control. Record the percentage of viable cells within the total gated population.

7. Calculation

Viability (%) = (Number of 7-AAD-negative events / Total gated events) x 100. Report the result to one decimal place.

8. Acceptance Criteria

Parameter	Criterion (example)
Minimum events acquired	≥ 10,000 gated cells
Unstained control	< 2% signal above the negative boundary
Reportable viability	Per product specification (e.g., ≥ 70%)

9. Deviations

Any departure from this procedure, or any result outside the acceptance criteria, shall be documented and investigated in accordance with the site deviation-management SOP prior to release of the result.

10. Revision History

Version	Description of Change	Effective Date
1.0	Initial issue.	—
2.0	Added unstained-control acceptance criterion; clarified minimum event count and gating steps.	—