Determination of Cell Viability by 7-AAD Exclusion Using Flow Cytometry

SOP-QC-0142 · Version 1.0 (DRAFT) · Quality Control — Analytical · GMP / 21 CFR 211, data integrity per ALCOA+

1. Purpose

To describe the procedure for quantifying cell viability in cell-therapy drug product using 7-aminoactinomycin D (7-AAD) exclusion measured by flow cytometry.

2. Scope

Applies to in-process and release viability testing of cell-therapy products by the QC Analytical group. Excludes viability by trypan blue and automated cell counters, which are covered under separate procedures.

3. Responsibilities

- QC Analyst — executes the procedure, records results contemporaneously, and reports out-of-specification results.
- QC Reviewer — performs the second-person review of data and calculations per data-integrity requirements.
- QC Supervisor — approves results and dispositions out-of-specification investigations.

4. Materials & Systems

- Calibrated flow cytometer qualified for the viability application
- 7-AAD viability staining solution (record lot and expiry)
- Stain buffer (PBS + 2% FBS or equivalent), 2–8 °C
- Single-use sterile tubes, pipettes, and tips
- Validated acquisition/analysis software with audit-trail enabled

5. Procedure

1. Confirm the flow cytometer has passed daily QC and is within its qualification interval; record instrument ID and QC status.
2. Record the 7-AAD reagent lot number and expiry; confirm it is within date.
3. Prepare a single-cell suspension and adjust to the target concentration specified in the batch record.
4. Transfer the specified cell volume to a labeled tube; prepare an unstained control in parallel.
5. Add the validated volume of 7-AAD staining solution to the test tube; do not add stain to the unstained control.
6. Incubate protected from light for the validated time at the specified temperature.
7. Acquire the unstained control first to set the negative gate, then acquire each test sample, collecting the specified minimum event count.
8. Gate the viable (7-AAD-negative) population using the strategy defined in the method; export the % viable result.
9. Record the result contemporaneously in the batch record and route data for second-person review.

6. Acceptance Criteria

- Minimum acquired events meet or exceed the method-defined threshold.
- Unstained control confirms correct negative-gate placement before test acquisition.
- Viability result is reported to one decimal place with the associated specification.

7. References

- Method: Flow Cytometry Viability Method (7-AAD) — [method number]
- SOP: Operation and Daily QC of the Flow Cytometer — [SOP number]
- Data Integrity Policy — [policy number]

8. Revision History

Version	Date	Summary of Change
1.0 (DRAFT)	Pending	Initial issue.

QC Review Record

#	Category	Review Item	Status
1	Completeness	All required sections present (Purpose, Scope, Responsibilities, Materials, Procedure, Acceptance, References, Revision Histo	PASS
2	Completeness	Document ID, version, and effective-date fields populated.	REVIEW
3	Clarity	Each procedure step is a single, concrete action written in the imperative.	PASS
4	Clarity	No undefined acronyms on first use.	PASS
5	Compliance	Second-person review step is explicitly required in the procedure.	PASS
6	Data Integrity (ALCOA+)	Contemporaneous recording of results is specified.	PASS
7	Data Integrity (ALCOA+)	Reagent lot/expiry and instrument ID/QC status are captured (attributable, original)	PASS
8	Human Decision Required	Confirm validated stain volume, incubation time/temperature, and minimum event count against the approved method before iss	HUMAN DECISION
9	Human Decision Required	Verify the exact specification/acceptance limit for viability for this product.	HUMAN DECISION
10	References	Referenced method/SOP/policy numbers are placeholders and must be completed	HUMAN DECISION

Status: DRAFT — NOT approved. Human QC items outstanding.
Reviewed by: Kalif Shear **Date:** _____