Utilizing flow cytometry within a quality control process for an iPSC-based manufacturing facility

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Abstract

Flow cytometry is a valuable technique for the analysis of induced pluripotent stem cells (iPSCs) and iPSC-derived cells within a quality-driven manufacturing environment. In our iPSC production facility, we utilize flow cytometry to assess iPSCs for key pluripotency markers and to characterize iPSC-derived cells as they mature to precursor, and then to endpoint cells such as microglia and cardiomyocytes. Additionally, this data and the pass/fail rate are used as a measure of manufacturing consistency and production success.

Here we present data from the Guava® easyCyte™ and Attune NxT flow cytometers, providing examples of pass and fail data. We note comparable results with the two different instruments, but with greater multiplexing and higher throughput potential when using the Attune.

Methods

Attune NxT flow cytometer (left) from Thermo Fisher Scientific and Guava® easyCyte™ cytometer (right) from Merk Millipore.

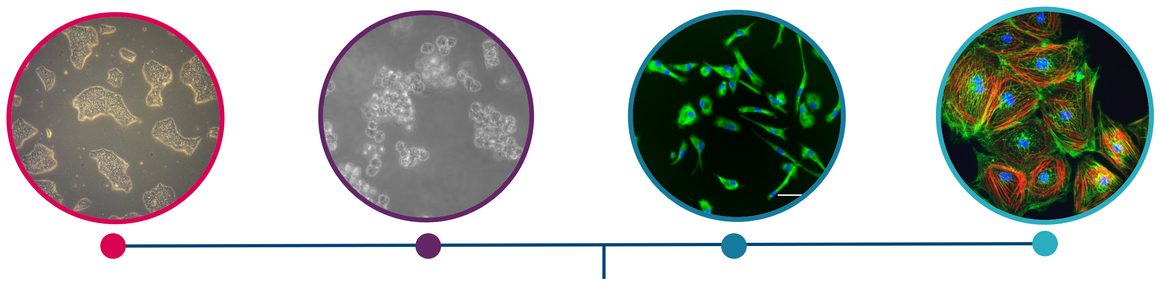
Cells of interest are either processed live or the cells are fixed with 4% PFA before antibodies and isotypes are conjugated to the cells. The samples are then run on either the Guava® easyCyte™ or Attune NxT flow cytometers and analyzed for key marker expression, based on the gating made using the antibody-relative isotype or the control sample.

Utilization of flow cytometry

1. Characterization of iPSCs Pluripotency markers measurement to ensure high quality starting material

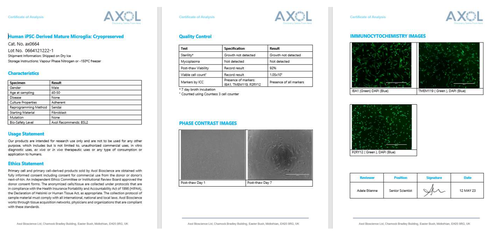
2. Analysis of precursor cells Measurement of key lineage-specific markers to ensure commitment to cell type

3. Characterization of endpoint cells Measurement of key cell markers to characterize endpoint cells including microglia and cardiomyocytes.



Quality control (QC) process:

- Establishment of pass threshold
- Assessment of cells by flow cytometry
- Analysis – pass / fail
- Reporting within quality management system and CoA



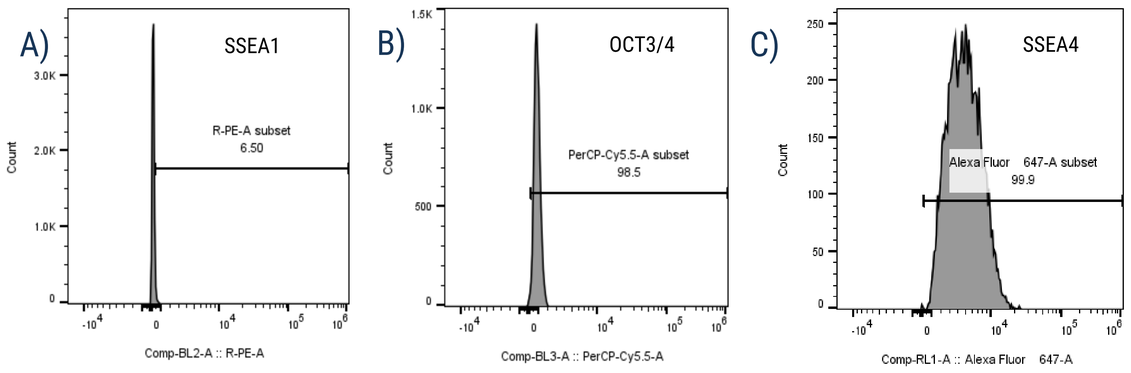
Results

1. Assessment of iPSCs for key pluripotency markers

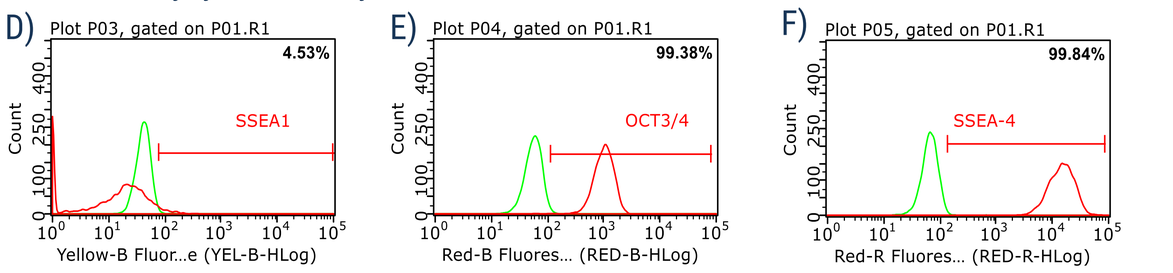
Establishment of pass / fail criteria

	SSEA1	Oct 3/4	SSEA4	Tra160	Nanog
Pass Threshold	<10%	>80%	>80%	>80%	>80%

Attune NxT flow cytometer



Guava® easyCyteTM flow cytometer

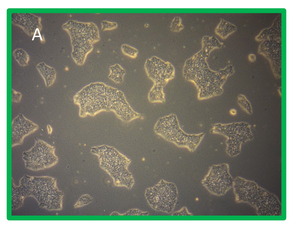


Example pass sample

	SSEA1	Oct 3/4	SSEA4	Tra160	Nanog
Attune	6.50%	98.50%	99.90%	99.30%	98.40%
Guava	4.53%	99.38%	99.84%	99.49%	98.69%

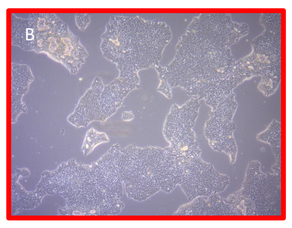
Figure 2. iPSC flow cytometry using the Attune NxT and Guava® easyCyte™. The results demonstrate the percentage of expression after gating using the respective isotype for each marker. **A)** SSEA-1 results analyzed from the Attune. **B)** OCT 3/4 results analyzed from the Attune. **C)** SSEA-4 results analyzed from the Attune. **D)** SSEA-1 results analyzed from the Guava with the antibody in red and the isotype in green. **E)** OCT3/4 results analyzed from the Guava easyCyteflow with the antibody in red and the isotype in green. **F)** SSEA-4 results analyzed from the Guava with the antibody in red and the isotype in green. SSEA-1 is used as a negative control, as a marker of spontaneous differentiation.

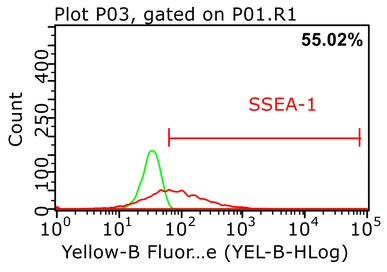
Example of pass result



A) Example of iPSC material demonstrating appropriate morphology, with round edges and uniform colonies. This batch would be expected to pass flow cytometry QC.

Example of fail result





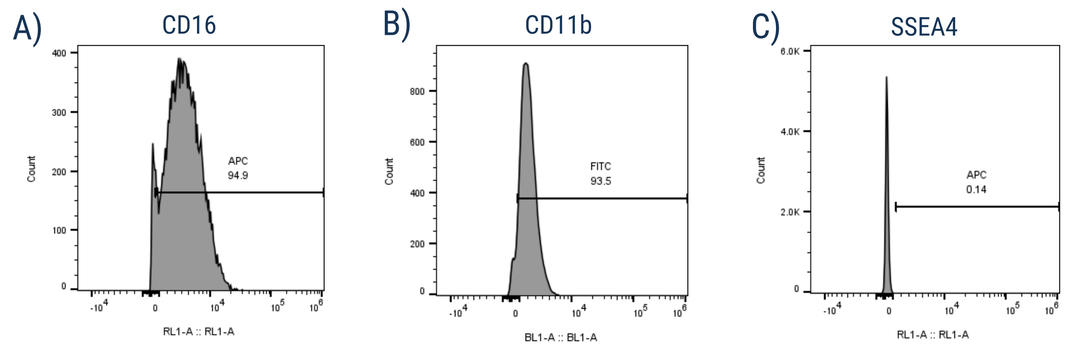
B) Left: Example of externally sourced iPSC material exhibiting aberrant morphology (non-uniform colonies with clustering at the top left). **Right:** Flow cytometry demonstrating SSEA-1 expression over threshold levels, therefore failing flow cytometry QC. This demonstrates the utility of flow cytometry analysis as part of our axoServices™ offering to external groups, which can identify issues with client starting material, maximizing project success.

2. Assessment of precursor cells: iPSC-derived monocytes

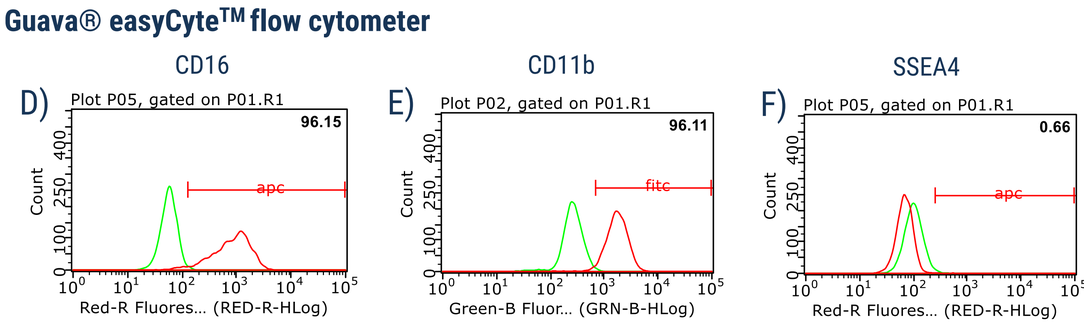
Establishment of pass / fail criteria

	CD14	CD16	CD11b	SSEA-4	CD163	CD206
Pass Threshold	>80%	>80%	>80%	<10%	>80%	>80%

Attune NxT flow cytometer



Guava® easyCyte™ flow cytometer



Example pass sample

	CD14	CD16	CD11b	SSEA-4	CD163	CD206
Attune	93.20%	94.90%	93.50%	0.14%	90.60%	90.90%
Guava	97.10%	96.15%	96.11%	0.66%	94.01%	87.59%

Figure 4. Monocyte flow cytometry of monocytes (microglia precursors) using the Attune NxT and Guava® easyCyte™ flow cytometers. The results shown are after gating using the respective isotype for each marker and represent the percentage of expression. The sample has met the criteria for all of the markers across both flow cytometers **A)** CD16 results analyzed from the Attune. **B)** CD11b results analyzed from the Attune. **C)** SSEA-4 results analyzed from the Attune. **D)** CD16 results analyzed from the Guava with the antibody in red and the isotype in green. **E)** CD11b results analyzed from the Guava with the antibody in red and the isotype in green. **F)** SSEA-4 results analyzed from the Guava with the antibody in red and the isotype in green. SSEA-4 is used a negative control (pluripotency marker).

Example fail result: immature monocytes

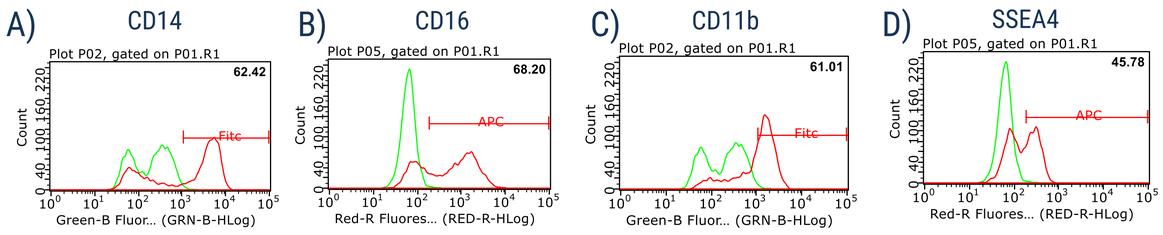


Figure 5. Monocyte flow cytometry results from the Guava easyCyteflow demonstrating a mixed mature and immature population within the sample which can be seen by a double peak in all of the markers. Therefore, the cells are not yet collectively at the threshold to proceed onto maturation to microglia. **A)** CD14 results. **B)** CD16 results **C)** CD11b results **D)** SSEA4 results.

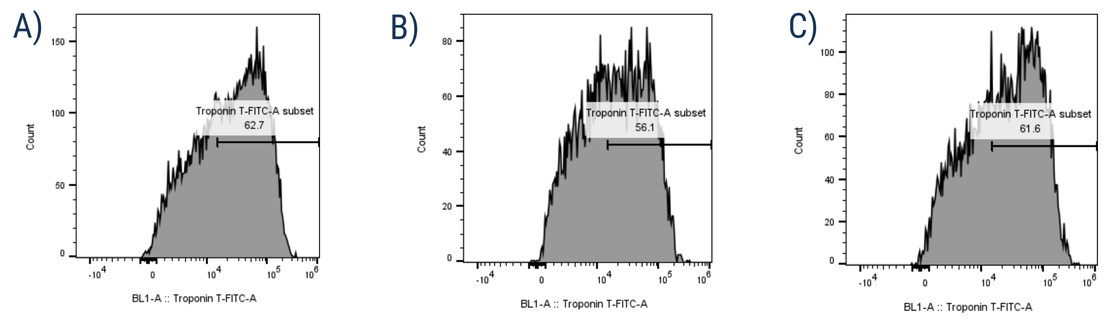
3. Assessment of endpoint cells: axoCells™ cardiomyocytes

Establishment of pass / fail criteria

	Cardiac Troponin T
Pass Threshold	>60%

Attune NxT flow cytometer

axoCells cardiomyocytes



iPSCs

Example pass sample

	Cardiac Troponin T
axoCells cardiomyocytes	60.13%
iPSCs	1.62%

Figure 6. Flow cytometry of axoCells cardiomyocytes using ax2508 cardiomyocytes on the Attune NxT flow cytometer. The graphs show comparative expression of Cardiac Troponin T from axoCells cardiomyocytes and iPSCs.

A) Cardiomyocyte Cardiac Troponin T expression repeat 1. **B)** Cardiomyocyte Cardiac Troponin T expression repeat 2. **C)** Cardiomyocyte Cardiac Troponin T expression repeat 3. **D)** iPSC Cardiac Troponin T expression repeat 1. **E)** iPSC Cardiac Troponin T expression repeat 2. **F)** iPSC Cardiac Troponin T expression repeat 3. The results table demonstrates the mean percentage expression, N=3.

Conclusion

Flow cytometry is a valuable asset within our overall manufacturing QC process, both to monitor manufacturing consistency and to ensure the quality of iPSCs and iPSC-derived cells. Building strict pass thresholds into our quality procedures (and ultimately, the certificate of analysis) enables us to consistently produce high-quality iPSC-derived cells to fuel disease models and compound screening at scale.

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