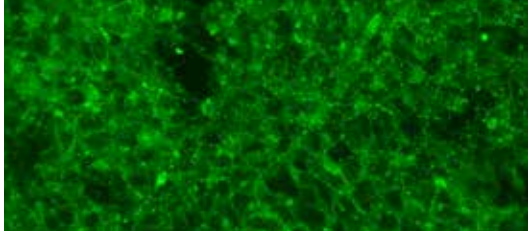
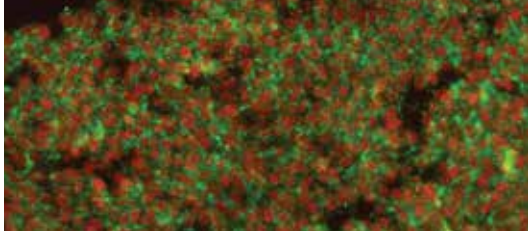
THE ESSENTIALS OF
LIFE SCIENCE RESEARCH
GLOBALLY DELIVERED™





ATCC®

ASSURING PLURIPOTENCY IN ATCC iPSCs

ATCC® induced pluripotent stem cells (iPSCs) are highly characterized and authenticated using immunological, phenotypic, genotypic and sterility-based analyses. ATCC scientists perform immunocytochemistry and flow cytometry to verify the presence of the appropriate markers of pluripotency. To confirm the pluripotent status, the colony morphology and karyotype of the iPSCs are examined. Finally, the cells are authenticated by short tandem repeat (STR) and tested for microbial contamination.

Characterization Assays Used by ATCC

IMMUNOCYTOCHEMISTRY

Immunocytochemistry is an accessible method for most laboratories to assess the pluripotency of iPSC cultures. The cells are fixed and then incubated with primary antibodies directed against pluripotency markers. These antibodies are then detected with a secondary fluorophore-conjugated antibody. Undifferentiated iPSCs can be characterized by the expression of pluripotent markers such as Nanog, Tra-1-60, SSEA4, and Tra-1-81. (Figure 1).

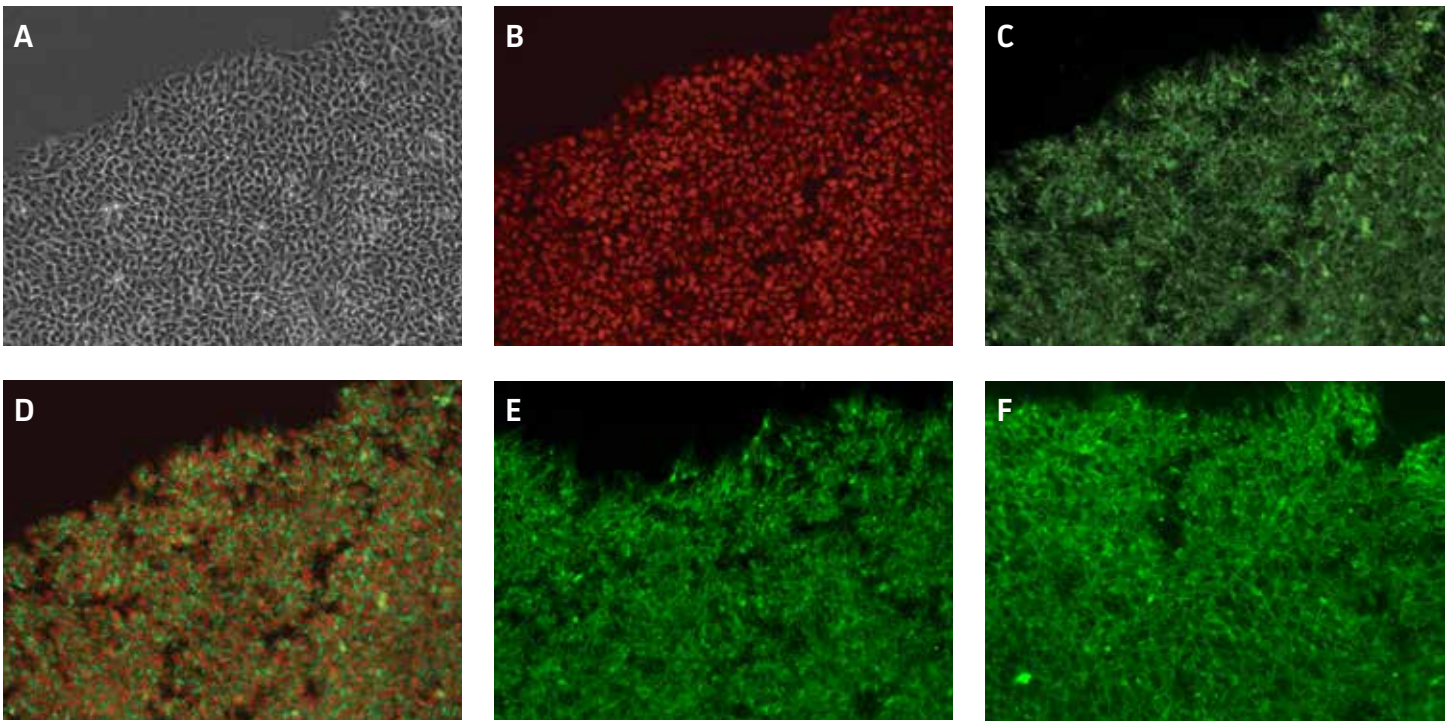


Figure 1. iPSCs express markers of pluripotency. Panel A) represents a phase contrast micrograph of iPSCs at 10x magnification. Panels B) through F) indicate iPSCs stained with antibodies directed against: B) Nanog, C) Tra-1-60, D) overlay of B) and C), demonstrating the simultaneous expression of a Yamanaka factor and a marker of pluripotency, E) SSEA4, and F) Tra-1-81.

FLOW CYTOMETRY

To quantitate the percentage of undifferentiated iPSCs in culture, flow cytometric analysis of pluripotency markers and differentiation markers is performed. Samples of cells are stained with a fluorophore-conjugated antibody directed against the pluripotency surface markers of interest. The cells are then counted by a flow cytometer. Upon differentiation, Tra-1-60, and SSEA4 expression levels decrease while SSEA1 expression increases. For an undifferentiated iPSC culture, the expression level of SSEA4 and Tra-1-60 pluripotency markers should be greater than 85% of the total cells and the expression level of SSEA1 should be less than 15% of the total cells (Figure 2).

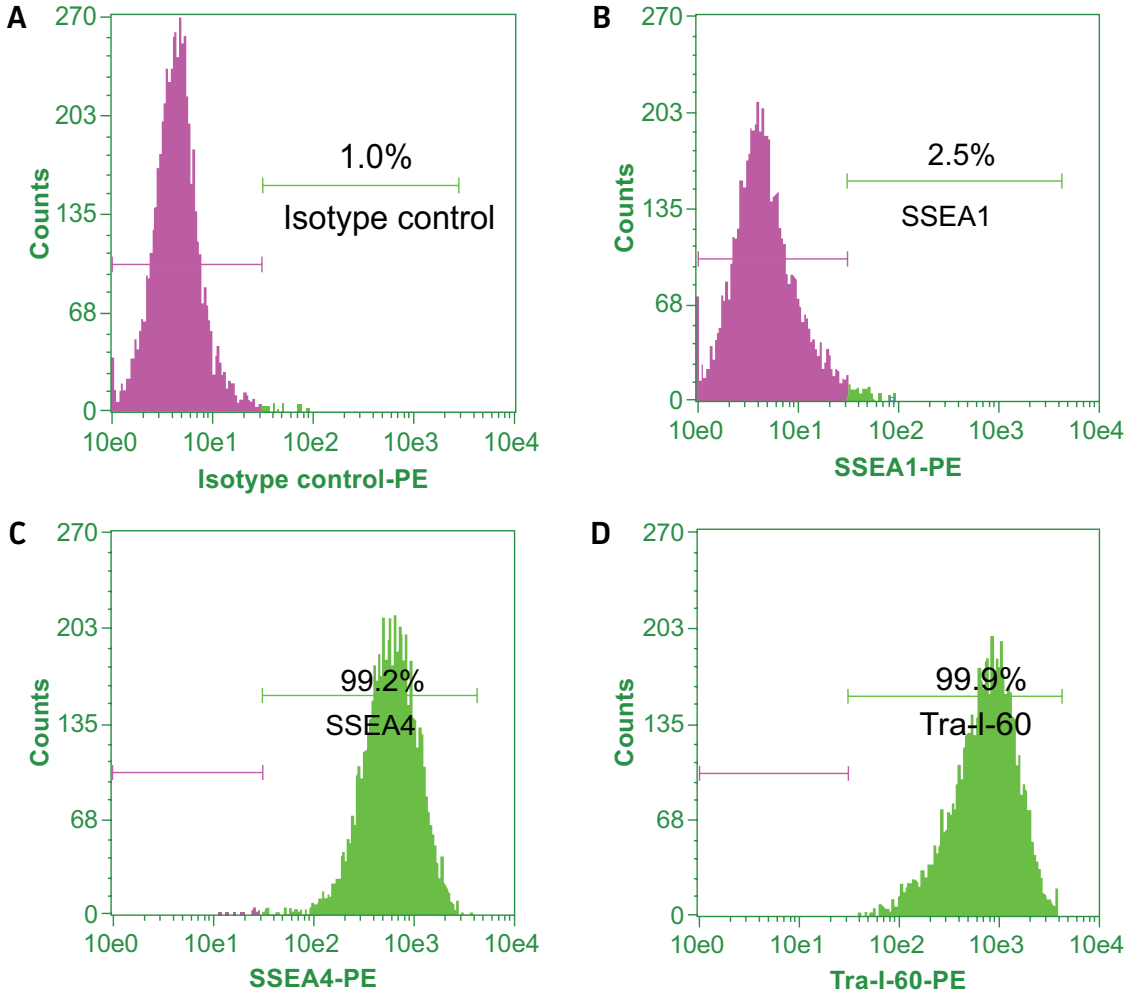


Figure 2. Flow cytometric analysis of pluripotency marker expression in iPSCs. The cells were stained with antibodies directed against A) isotype control-PE, B) SSEA1-PE, C) SSEA4-PE, and D) Tra-I-60-PE.

In addition to the aforementioned methods of quality control, ATCC recommends karyotyping and STR analysis of iPSCs after several passages. Undifferentiated iPSCs grow as compact colonies and exhibit high nucleus-to-cytoplasm ratios and prominent nucleoli (Figure 3). When cultured in Pluripotent Stem Cell SFM XF medium (ATCC No. ACS-3002), small iPSC colonies may initially exhibit loose colony morphology but will become more compact as the colony grows larger. Please download a copy of the ATCC Stem Cell Culture Guide at www.atcc.org/guides for more information on the quality control and characterization of ATCC iPSCs.

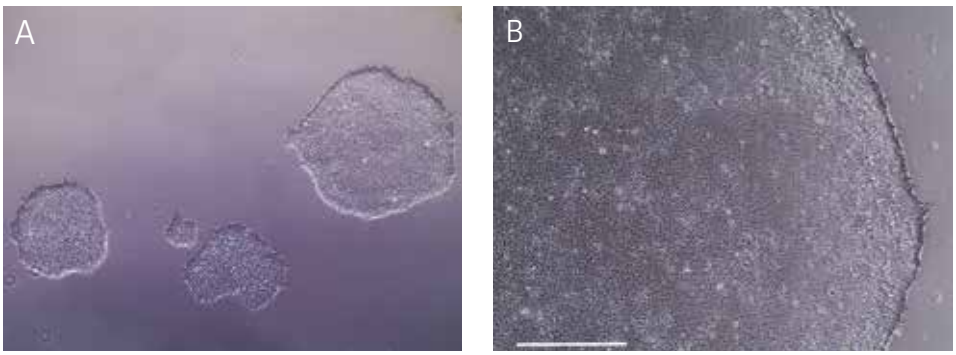


Figure 3. Characteristic morphology of iPSCs grown in feeder-free cultures. Phase contrast micrographs of iPSC colonies at A) 4x magnification and B) 10x magnification indicate the compact colony morphology of undifferentiated iPSCs.

SAMPLE CERTIFICATE OF ANALYSIS FOR ATCC IPSCs

For each iPSC culture, ATCC provides a Certificate of Analysis that documents the pluripotent status, reprogramming method, passage number, and viability of an individual lot. ATCC also includes authentication data such as STR, viral testing, sterility testing, and karyotype. In some cases, ATCC provides information such as the donor's disease status, age, ethnicity, and gender. Please see the sample Certificate of Analysis below:

ATCC®

CERTIFICATE OF ANALYSIS

ATCC® Number: **ACS-1021™**
Lot Number:

Name or Designation: **ATCC-CYS0105 Human Induced Pluripotent Stem Cells**
Parental Cell Type: **Primary cardiac fibroblast**
Passage No.: **P16**
Reprogramming Method: **Sendai viral expression of Oct4, Sox2, Klf4, and Myc genes**
Disease: **Normal**
Depositor/Institution: **ATCC**
Depositor Designation: **N/A**
Donor Information: **72 years old, Caucasian male**
Product Format: **Cells cryopreserved in the appropriate cryopreservation medium**
Expiration Date: **Not applicable**
Storage Conditions: **Vapor phase of liquid nitrogen**

Test Description	Method	Specification	Result
Post-Thaw Viable Cell Recovery	hiPSC culture	≥ 30 colonies in 5 days	Pass Number of colonies: 192
Surface Antigen Expression of Stem Cell Markers	Flow cytometry	SSEA4, Tra-1-60 (expressed on undifferentiated hiPSCs) > 85% SSEA1 (expressed on differentiated hiPSCs) < 15%	Pass Marker: Result: SSEA4 96.86% Tra-1-60 93.29% SSEA1 1.37%
Germ Layer Differentiation	EB formation and qRT-PCR analysis	Exhibits increased expression of a gene from endoderm, mesoderm, and ectoderm layers, relative to pluripotent cells of the same lineage	Germ Layer: Gene: Fold Induction: Endoderm AFP 7630.39 Mesoderm Runx1 18.34 Ectoderm Pax6 6.97
Plasmid Integration	PCR	None detected	Pass None detected
Sterility (Bacterial and Fungal Testing)	Growth on agar	No growth after 21 days	Pass No growth

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E-mail: tech@atcc.org
or contact your local distributor

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CERTIFICATE OF ANALYSIS

ATCC® Number: **ACS-1021™**

Lot Number:

Mycoplasma	Direct culture and Hoechst DNA staining	None detected	Pass None detected
Identity	STR[1]	Consistent with expected	Pass D5S818: 9, 13 D13S317: 11, 12 D7S820: 10, 11 D16S539: 10, 12 vWA: 17, 19 TH01: 6, 9.3 Amelogenin: X, Y TPOX: 9, 11 CSF1PO: 12
Karyotype	G banding	Normal karyotype, 46 XY	Pass
Viral Panel Testing	PCR	None detected when assayed for CMV, EBV, HepB, HIV1, and HPV	Pass

1 – STR results are compared to the STR profile of the material of origin. If the material of origin is a donor, then the STR profile testing serves as a baseline result for future lots of material.

Manager of Material Release; Quality Assurance

ATCC hereby represents and warrants that the material provided under this certificate is pure and has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and correct to the best of the company's knowledge and belief. This certificate does not extend to the growth and/or passage of any living organism or cell line beyond what is supplied within the container received from ATCC.

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