SARTORIUS

White Paper

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Assuring Pluripotency of ESC and IPSC Lines

Introduction

Compared to other cell types, stem cells have the unique ability to "self-renew" their populations by dividing into identical daughter stem cells as well as a capacity for "differentiation" into more specialized cell types. In this way, stem cells provide the body's foundation during embryogenesis and facilitate tissue growth and repair later in life. However, stem cells demonstrate different degrees of differentiation potency. Pluripotent stem cells are of particular interest in clinical and developmental biology research as these cells have the capacity to specialize into cell types originating from the three germ layers (i.e., ectoderm, mesoderm, and endoderm) that go on to form the entire body.

Aside from their differentiation capacity, pluripotent stem cells are also characterized by the expression of particular stem cell markers, alkaline phosphatase activity, and the capacity to form benign tumors comprised of tissues from all three germ layers, as can be seen in teratoma formation in vivo assays and Embryoid bodies (EBs) formation in vitro.

While embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs)—are both regarded as pluripotent, these cell types arise from different origins. ESC lines are established from the cells of the inner cell mass of a preimplantation day-5 blastocyst. iPSC lines, on the other hand, arise from somatic cells that have been genetically reprogrammed to a stem cell-like state.

Pluripotency and a normal karyotype should be confirmed in newly established ESC and iPSC lines before use. When properly maintained, human pluripotent stem cells can self-renew in culture and provide a nearly unlimited pluripotent cell source. However, improper culture and maintenance of pluripotent stem cells—such as colony overgrowth, poorly formulated culture medium, contamination, or premature passaging—will cause cultures to spontaneously differentiate into undesired cell types. As such, pluripotency should also be verified in unfamiliar lines, or lines that have spent time in extended storage. After initial confirmation of pluripotency, cultures should still be assessed at regular intervals as pluripotent cells will begin to spontaneously differentiate and|or acquire karyotype abnormalities at higher passage numbers. For the best endpoint of differentiation efficiency, maintaining an initial pluripotent state is critical for success. In the following sections we will give an overview of several accepted methods to confirm pluripotency.

Methods to Confirm Pluripotency in Newly Established and Existing Pluripotent Stem Cell Lines

While changes in morphology i.e., a shift from tight, compact colonies (Fig. 1) to loose, uneven colonies (Fig. 2) can visually suggest loss of pluripotency in cultures, additional assessments should be performed to confirm pluripotency status.

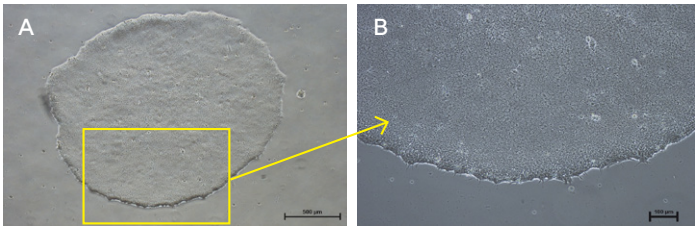


Figure 1: Examples of tight, compact morphology observed in pluripotent hESC and hiPSC cultures. A (X40), B (X100)

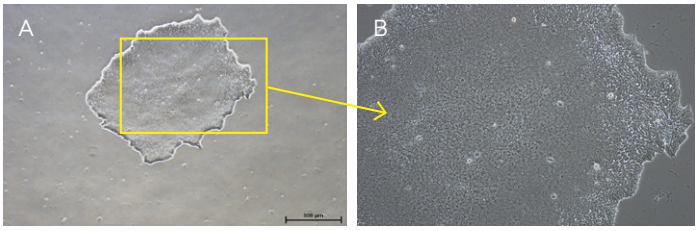


Figure 2: Examples of loose, uneven morphology observed in pluripotent hESC and hiPSC cultures. A (X40), B (X100)

Enzymatic Activity

The blastocyst inner cell mass (ICM) is additionally characterized by elevated alkaline phosphatase (ALP) presence and activity[1]. As the pluripotent cells within the ICM begin to specialize into specific cell types, ALP levels decrease. As follows, pluripotency may also be confirmed by measuring ALP activity and presence. Colorimetric lysate-based assays that measure ALP enzymatic activity and ALP presence, respectively, are commercially available.

ESC and iPSC show High Alkaline Phosphatase Activity.

30 minutes | **Alkaline Phosphatase (ALP)**

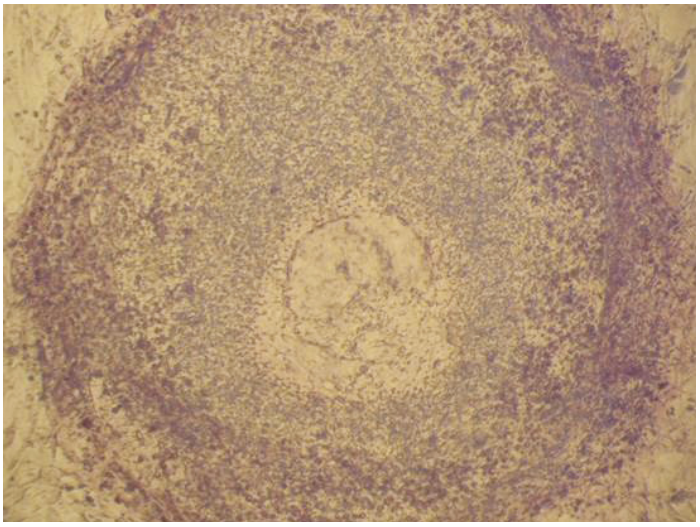


Figure 3: Alkaline phosphatase assay of an iPSC colony

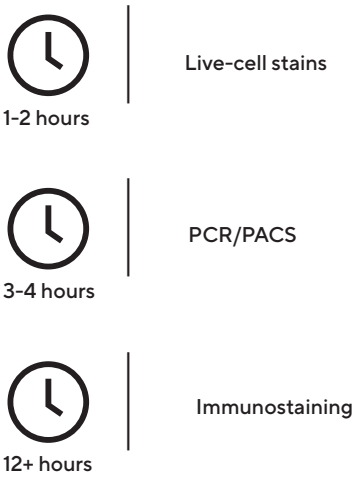
Genetic and Proteomic Markers

OCT4, SOX2, and NANOG form the core factors responsible for supporting pluripotent stem cell self-renewal and are highly expressed in pluripotent stem cells[2]. During cellular specialization, these factors are rapidly downregulated. Pluripotent stem cells are reported to also express the cell surface markers SSEA4 and Tra-1-60 [3], which can additionally be employed in pluripotency verification assessments.

When using markers to confirm pluripotency, it is imperative to check at both the genetic and proteomic levels. Genetic expression of pluripotency markers may be verified by traditional PCR or qPCR measurement of pluripotency factors. Immunostaining (Fig. 4), fluorescence-activated cell sorting (FACS), and livestaining can confirm the presence of pluripotency factors at the proteomic level.

Immunostaining and FACS approaches are best when a large quantity of cells is available for assessment and can be used with both intracellular and cell surface markers. A FACS-based approach is also useful if quantification of pluripotent cells is required. When only a small quantity of cells is available or when checking for reprogrammed cells to establish an iPSC line, non-permanent live cell stains such as CDy1 offer the best option for early pluripotency confirmation.

Immunofluorescence can be used to confirm pluripotency.



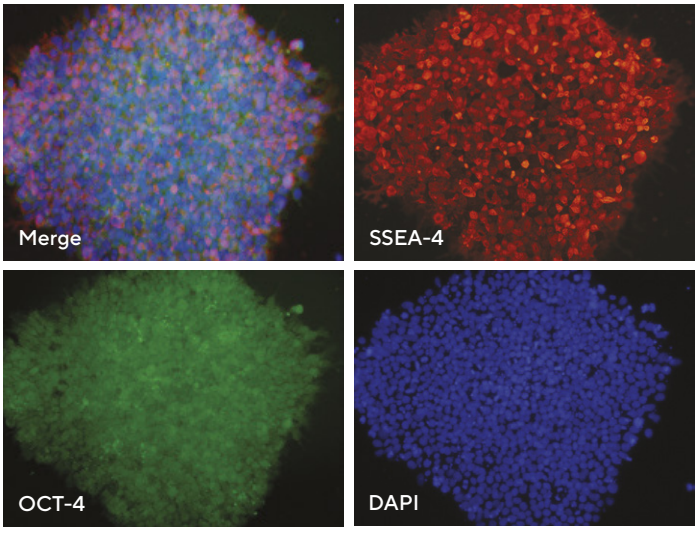


Figure 4: Immunofluorescence can be used to confirm pluripotency. Here, hESC H1 line is assessed for expression of SSEA4 and OCT4 pluripotency markers.

Functional Assessment

Teratoma Assay

Genetic and proteomic assessments are beneficial as both provide an early and swift indication of colony potency. However, pluripotency validation via functional assessment is also an important consideration as it confirms proper pluripotency performance. Functional assessments test the pluripotent cells' ability to form all the cell types of the body. One such approach is to inject the cells into an immunodeficient mouse either intramuscularly, subcutaneously, under the testis capsule, or under the kidney capsule[4]. If the cells are truly pluripotent, they will produce a teratoma (Fig. 5), or benign tumor comprised of tissue types from all three germ layers. Following injection, cells are allowed to remain undisturbed at the implantation site for approximately 6-12 weeks or until the teratoma—if successfully produced—has reached an appropriate size for sectioning and histological analysis. Histological preparations may be done intramurally or commercial services may be used for preparation. Presence of all three germ layers is confirmed by morphological assessment and|or immunohistochemistry analysis with germ layer appropriate markers such as SOX1 (ectoderm), Brachyury (mesoderm), and GATA6 (endoderm)[5].

The Teratoma Assay Is Useful in Confirming Cell Culture Pluripotency.

12 weeks | Teratoma

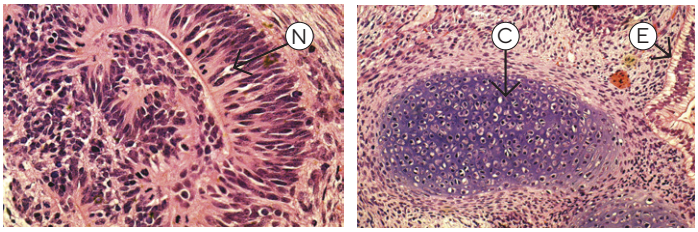


Figure 5: The teratoma assay is useful in confirming cell culture pluripotency. Here, hESC line H9.2 cells were injected intramuscularly into the hind leg of immunocompromised SCID-Beigemice. After 12 weeks, the teratoma was removed, sectioned, and stained with H&E. Histological assessment confirmed tissue from all three germ layers: ectoderm (neural rosette, N), mesoderm (cartilage, C), and endoderm (endoderm columnar epithelium, E).

Embryoid Body (EB) Formation

While effective, teratoma assays can be time-consuming and require special training and accommodations for animal use and experimentation work. A swifter alternative is the assessment of embryoid body (EB) formation (Fig. 6). Embryoid bodies are formed following forced aggregation of single pluripotent cells in suspension. If the cells are truly pluripotent, the EBs will spontaneously differentiate into cell types of all three germ layers. EBs are typically produced by either static suspension or the hanging drop method. Static suspension requires the cells under study to be made into a single-cell suspension that is subsequently placed in a low adherence bacterial petri dish. In the dish, individual cells randomly form aggregates of varied sizes. The hanging drop method also requires a single cell suspension of a defined cellular concentration. Here, uniform droplets of suspension with identical cell numbers are allowed to hang from the lid of a petri dish. Gravity pulls the individually suspended cells to the tip of the drop to form evenly-sized aggregates. Once the EBs have formed, they are allowed to continue to spontaneously differentiate in culture until they have reached a size suitable for sectioning. Once sectioned, EBs are then morphologically and|or immunohistochemically assessed to confirm culture pluripotency.

Embryoid Body (EB) Formation Is an Acceptable Alternative to the Teratoma Assay.

2 weeks | Embryoic Bodies (EB)

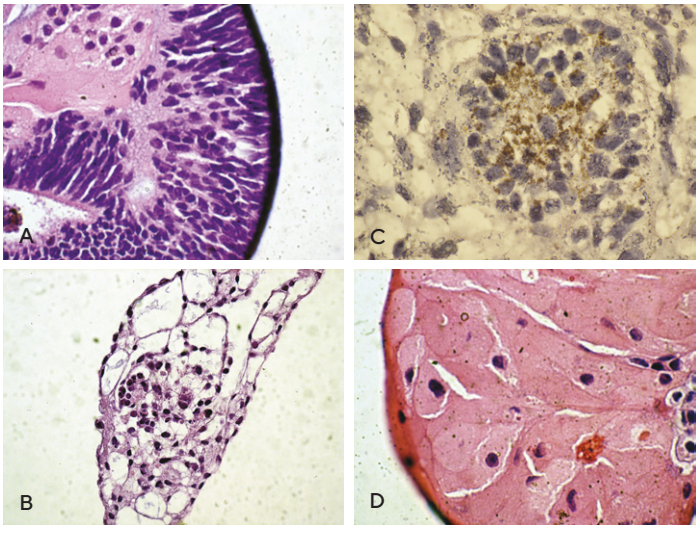


Figure 6: Embryoid body (EB) formation is an acceptable alternative to the teratoma assay. Here, hESC line H9.2 cells were allowed to form EBs in static suspension for 14 days. EBs were sectioned and stained with H&E. Histological assessment confirmed ectoderm (neural rosette, A and B) and mesoderm (primitive blood vessels, C; megakaryocytes, D) cell types.

Summary

Proper maintenance of cell pluripotency is critical for experimental differentiation performance, and for the validation of hPSCs. Newly established, received, or thawed ESCs and iPSCs should be tested for pluripotency using the methods previously described. Checking the karyotype is also important as abnormal karyotypes in pluripotent stem cell lines may significantly impact culture behavior and differentiation efficiency. Upon confirmation of pluripotent status and genetic structural integrity, early passages of cell cultures should be expanded and frozen down to ensure prolonged maintenance of the line. Avoiding colony overgrowth and using a reliable maintenance medium such as NutriStem® hPSC XF (Fig. 7) will assist prolonged maintenance of culture pluripotency. While colony morphology itself may suggest culture potency, lines should still be checked at regular intervals as higher passages have a greater chance of pluripotency loss and karyotype aberrations.

Figure 7: Always use a reliable maintenance medium such as NutriStem® hPSC XF Medium to ensure long-term pluripotency preservation.

Product	Cat.#	Size
NutriStem® hPSC XF Medium	05-100-1A	500mL

References

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