

Resource Summary Report

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MAINTENANCE CULTURE OF iPSCs (Basic Protocol 1)

DOI:10.17504/protocols.io.48vgzw6

Type: Protocol

Proper Citation

Michael S. Fernandopulle, Ryan Prestil, Christopher Grunseich, Chao Wang, Li Gan, Michael E. Ward 2019. MAINTENANCE CULTURE OF iPSCs (Basic Protocol 1). protocols.io dx.doi.org/10.17504/protocols.io.48vgzw6

Protocol Information

URL: <https://dx.doi.org/10.17504/protocols.io.48vgzw6>

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Group: Neurodegeneration Method Development Community

Summary: Human iPSCs are an ideal system for studying human biology due to their rapid proliferation, genomic stability, and ability to differentiate into many somatic cell types. Historically, specialized culture practices and costly reagents have hindered widespread adoption of iPSCs by the cell biology community. In recent years, however, development of new culture techniques and improved media formulations have dramatically simplified iPSC culture and reduced costs. The protocols described in this unit are adapted from a collection of publications that establish optimal practices for the maintenance of human iPSC cultures (Beers et al., 2012; Ludwig et al., 2006; Chen et al., 2011). While these publications provide useful guidelines for the stem cell novice, here we distill the fundamental procedures necessary for maintaining iPSCs in a pluripotent state and highlight critical steps that may need to be optimized for individual applications. In practice, iPSC lines of interest are usually maintained in an undifferentiated state in small cultures (1 to 3 wells of a 6-well plate) to reduce reagent use before being expanded as needed for experimentation. Essential 8 (E8) is the simplest defined medium for hiPSC culture. E8 may be prepared from its components by the consumer (Table 1; Chen et al., 2011) or purchased as a preformulated kit. Other commercially available media may be substituted, such as mTeSR1 or StemFlex. A volume of 12 ml of medium should be added to each 10-cm tissue culture dish or distributed evenly across each standard multiwell plate (i.e., 2 ml/well for a 6-well plate). E8 should be aspirated and replaced with fresh medium daily, although a double volume may be added at low confluency to permit an extra day of culture without medium changes. StemFlex and E8 Flex contain components

that stabilize the recombinant growth factors present in the medium, permitting medium exchange every other day as a general practice. Some iPSC lines (e.g., WTC11) tolerate every-other-day medium changes of standard E8 medium without loss of pluripotency or cell death, further reducing costs of medium and consumables. Finally, mTeSR1 may promote cell survival in stressful conditions better than E8, especially for finicky iPSC lines, although supplementation with a ROCK inhibitor (RI) is also recommended in such scenarios. Use of standard E8 will be assumed throughout this basic protocol. Additionally, this protocol uses Matrigel-coated tissue culture plates. Matrigel works well for iPSC culture and has been widely adopted for research applications. However, since Matrigel is derived from murine sarcoma cells, it is not chemically defined and exhibits batch-to-batch variability. Alternative defined coatings include recombinant laminin or vitronectin, although these substrates are typically more costly. Notably, downstream neural differentiation described in these protocols occurs in fully defined conditions (see Basic Protocols 5 to 8), so the choice of iPSC substrate is of minimal scientific importance to all but clinical-grade applications.

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External URL: <https://doi.org/10.1002/cpcb.51>

Version: 1

Publication Date: 2019

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Record Creation Time: 20210329T220536+0000

Record Last Update: 20210329T221033+0000

Data and Source Information

Source: