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Good practices in hPSC handling

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We use this collection and it's working

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Abstract

This protocol collection provides a comprehensive and practical guide for the maintenance, passaging, freezing, and thawing of human pluripotent stem cells (hPSCs). Designed to support reproducibility and transferability, especially among new users, the protocols are informed by extensive experience from established hPSC core facilities.
In addition to detailed step-by-step procedures, the collection includes high-quality reference images illustrating healthy hPSC morphology under various culture conditions, as well as visual examples of spontaneous differentiation—serving as critical visual benchmarks for cell quality assessment. The protocols also cover the in-house preparation of Essential 8 (E8) medium and the use of key survival-supporting compounds, including ROCK inhibitors (ROCKi), CEPT cocktail, and CloneR2, ensuring enhanced cell viability during critical manipulations.
By consolidating expert-validated practices and making them accessible through a user-friendly format, this collection aims to serve as a foundational resource for laboratories working with hPSCs and to facilitate the standardization of stem cell culture techniques across different research environments.

Guidelines

- Always work aseptically in a Class II biosafety cabinet.
- Ensure that you keep the safety cabinet clean and tidy.
- For incubation, place culture vessels on trays to contain any accidental spillage of media and reduce the risk of contamination.

To avoid cross contamination between cell lines:

- Only one cell line at a time should be handled in a biosafety cabinet when feeding or passaging the cells.
- Bottles or aliquots of media should be prepared for separately for each cell line .
- Aliquots should be labelled with the name of the line and the date prepared and stored and handled appropriately.

This protocol collection describes the culture of hPSC under normoxia conditions (5%CO_2) due to availability in most cell culture laboratories. However, to reduce genomic instability during culture we recommend to incubate hPSC under **hypoxia conditions**: 5%CO_2 and 5% O_2.

Safety warnings

The estimated time for an individual protocol does not account for the time required to complete supporting protocols.

Before start

Before starting this protocol, ensure that you have all of the materials required to complete the protocol with ease and efficiency.

Files

SEARCH

Protocol

NAME

Maintenance of hPSC

VERSION 1

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Protocol

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Reference pictures of hPSC cultured in defined conditions

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E8 media production

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Survival factors for hPSC growth

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Coating of tissue Culture Vessels for hPSC

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Thawing of cryopreserved hPSC

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Non-enzymatic passaging of hPSC

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Single cell passaging of hPSC

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Manual picking and passaging of hPSC colonies

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Freezing of hPSC

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