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Maintenance of hPSC

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We use this protocol and it's working

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Disclaimer

The reported protocols are based on the authors experience, and may partially differ from the original protocols provided by the companies.

Abstract

This protocol describes maintenance of established hPSC lines in expansion media on matrix coated culture vessels.

Guidelines

Depending on the growth characteristics of specific hPSC line, cells must be passaged every 5-7 days in order to maintain log phase of growth and avoid induction of differentiation.
In general hPSC lines are maintained in a 6 well format, however information on other tissue culture vessel formats are also included in this protocol as a guidance.

Materials

LABORATORY EQUIPMENT AND CONSUMABLES

Use sterile material

- 1/5/10 mL serological pipettes
- 15/50 mL conical tubes
- Cell culture treated plastic vessels of choice e.g. 24, 12 or 6-well plates, T25, T75 flasks, 10cm dishes
- 10/200/1000µL tips and micropipettes (optional)
- Aspirator pump with disposable pipette
- Centrifuge
- Microscope, if available Stereo Microscope
- Disposable scraping tools (pipette tips, glass rods, small size cell scrapper, syringe and needle)
- Incubator at 37^{o}C and 5% CO_2 or under hypoxic conditions, 5% O_2/ 5% CO_2
- Class II Biosafety Cabinet

MEDIA AND REAGENTS

Vitronectin

Vitronectin (VTN-N) Recombinant Human Protein, Truncated **Thermo Fisher Catalog #**A14700

Vitronectin XF™ **STEMCELL Technologies Inc. Catalog #**#07180

Laminin

MACSmatrix Laminin 511 **Miltenyi Biotec Catalog #**130-136-454

rhLaminin-521 **Thermo Fisher Catalog #**A29248

CellAdhere™ Laminin-521 100 µg **STEMCELL Technologies Inc. Catalog #**77003

Basement Membrane Products

Geltrex™ LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix **Thermo Fisher Catalog #**A1413301

Matrigel hESC-qualified (Corning Cat# 354277) **Corning Catalog #**354277

Cell Culture Media

Essential 8™ Medium **Gibco - Thermo Fisher Scientific Catalog #**A1517001

Essential 8 Flex complete medium (E8) **Gibco - Thermo Fisher Scientific Catalog #**A2858501

StemFlex Medium **Thermo Fisher Scientific** **Catalog #**A3349401

mTeSR™1 500 mL Kit **STEMCELL Technologies Inc.** **Catalog #**85850

mTeSR plus media kit **STEMCELL Technologies Inc.** **Catalog #**100-0276

eTeSR **STEMCELL Technologies Inc.** **Catalog #**100-1215

StemMACS™ iPS-Brew XF human: Basal medium and supplement **Miltenyi Biotec** **Catalog #**130-104-368

Homemade Cell Culture Media: Essential 8 (E8) Medium (see protocol **E8 media production**)

Selection of culture conditions

15m

1 The following points should be considered when choosing hPSC culture conditions: 15m

- A number of media will work with an array of different matrices.
- The rate of growth of cell lines will differ between different matrix/media combinations and cell lines may exhibit a transient change in morphology until adapted to the new culture conditions.
- When transitioning cell lines between different media/matrix combinations follow the manufacturers guidance. Usual recommendations: do not switch the media and matrix at the same time. Make a gradual change of media including proportions until full transition.
- hPSC often require a couple of passages to adapt to the new culture conditions.
- Following transition to new culture conditions, perform quality control testing as appropriate for the intended use of the cells.
- For commercial media, prepare, store and use as directed by the manufacturer. For media made in house, use and store under the same conditions established for the validation of the media.

	A	B	C	D	E	F	G
	Media/Matrix	Truncated Vitronectin	Vitronectin XF	Matrigel	Geltrex	Laminin 521	Laminin 511
	Essential E8	XX	X	X	XX	X	
	Essential E8 Flex	XX	X	X	XX	XX	
	mTeSR1	X	XX	XX	X		
	mTeSR Plus		XX	XX			
	StemMACS iPSC-Brew XF			XX	XX	XX	XX
	Stem Flex			X	XX	XX	X

Table 2. Media and matrix possible combinations
XX = recommended by manufacturer and tested by CoreEuStem member
X = tested and recommended by CoreEuStem member
Homemade Cell Culture Media: Essential 8 (E8) Medium (see protocol **E8 media production**)

Note

The matrix media list is not exhaustive. It reflects the media and matrices that are used by the members of CorEuStem for their maintenance protocols.

2 Inspect hPSC cultures daily using a microsope to monitor morphology, the presence of spontaneous differentiated cells, and confluence. Based on these observations, determine if the cultures require further action (e.g. removal of differentiated cells, passaging). Use reference images in the protocol: **Reference pictures of hiPSC cultured in defined conditions** to guide your assessment of the cultures. Cell lines may vary in their growth rate and morphology in different media/matrix combinations. 15m

STEP CASE

Culture >70% confluent 1 step

Culture is >70% confluent and < 10% of the colonies in the culture appear differentiated upon visual inspection.

3 If the cells require passaging, refer to the protocol **Non-enzymatic passaging of hPSC** or **Single cell passaging of hPSC**. Otherwise performe a complete media change (refer to Culture <70% confluent tab).

Note

Assessing confluency is inherently subjective. Whenever possible, use imaging systems capable of calculating the surface area of the culture vessel occupied by cells to ensure a consistent and objective evaluation of cell culture confluency.

If such imaging tools are not available, utilize a collection of images captured during the culture process at various stages of confluency to train staff in recognizing and estimating different confluency percentages.

Keep in mind that the growth rate required to achieve confluency is influenced by both the specific cell line and the cell culture system used.

Protocol references

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