



EBiSC

Controlled Document

Standard Operating Procedure (Benchmark Protocol)

Number: SOP/REA/37

Version 1

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Document Details

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1. ***Introduction and Purpose***

- 1.1. This SOP describes the procedure for preparing and storing mTeSR1 media for iPSC maintenance.

2. ***Scope***

- 2.1. This procedure is intended as a 'Benchmark Protocol' for use by EBiSC derivation centres providing iPSC lines intended for the EBiSC Foundational Collection.
- 2.2. The scope of this SOP refers to the production of mTeSR1 cell culture media only.

3. ***Cross References***

- 3.1. These references relate specifically to the procedures detailed in this SOP.

Regulations:	None
Standards:	None
Guidelines:	None
Other:	None

4. ***Keywords***

- 4.1. Media, cell culture, iPSC, mTeSR.

5. ***Health and Safety***

- 5.1. Prior to handling laboratory chemicals refer to the relevant material safety data sheet to identify any potential hazards.
- 5.2. If this procedure involves handling human tissue, treat these as potentially hazardous, even if they have been tested, and found negative, for agents known to be transmitted in human tissue.

6. ***Documentation***

- 6.1 SOP/RDP/60 - Cryopreservation and Recovery Upon Thaw of iPS Cells



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- 6.2 SOP/RDP/61 - The Culture and Maintenance of Induced Pluripotent Stem Cells (iPSC)
- 6.3 SOP/RDP/62 - Chemical Passaging of Human Induced Pluripotent Stem Cells Using EDTA
- 6.4 SOP/REA/36 - The Preparation of Geltrex and Matrigel for the Maintenance of IPSC

7. *Materials*

7.1. *Equipment*

- 7.1.1. Class II Microbiological Safety Cabinet
- 7.1.2. Labelling System
- 7.1.3. Fridge
- 7.1.4. Freezer
- 7.1.5. Pipette gun

7.2. *Consumables*

- 7.2.1. mTeSR Basal Medium Kit (Stemcell Technologies 05850)
- 7.2.2. Pipettes
- 7.2.3. Bottles (Various sizes)
- 7.2.4. PBS (-/-)

8. *Procedure*

- 8.1. All media preparation procedures must be performed under aseptic conditions within in a class 2 safety cabinet.
- 8.2. Upon receipt, all media must be stored in accordance with manufacturer's instructions.
- 8.3. **mTeSR1 media**
 - 8.3.1. mTeSR1 supplement (5x-100mls) must be stored at $-20 \pm 5^{\circ}\text{C}$ upon receipt.
 - 8.3.2. mTeSR Basal media (400mls) must be stored at $5 \pm 3^{\circ}\text{C}$.



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- 8.3.3. When required, remove the mTeSR supplement (5x) from the freezer and thaw overnight at $5 \pm 3^{\circ}\text{C}$ prior to use.
- 8.3.4. Aseptically add 100ml of mTeSR supplement (5x) to 400mls of basal media using a stripette.
- 8.3.5. Mix the basal media and supplement together by gently pipetting up and down.
- 8.3.6. Aliquot media into volumes required for 1 culture week and label with mTeSR 1 'complete' and expiry date (see below).
- 8.3.7. Expiry date depends on storage conditions. Complete mTeSR1 may be stored at $5 \pm 3^{\circ}\text{C}$ for 1 week or may be stored at $-20 \pm 5^{\circ}\text{C}$ for 6 months.
- 8.3.8. Frozen mTeSR complete may be thawed once. Do not repeatedly freeze thaw this media.
- 8.3.9. Thaw complete mTeSR 1 media at $5 \pm 3^{\circ}\text{C}$ overnight. The thaw date must be written on the media bottle.
- 8.3.10. Prior to use, warm mTeSR1 to ambient temperature. Do not leave media at room temperature for longer than 2 hours per day and avoid exposure to light. After this time mTeSR 1 complete must be returned to the fridge at $5 \pm 3^{\circ}\text{C}$. mTeSR 1 complete may be kept for 1 week under these conditions and must be discarded after this week.

END OF SOP