



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 $5 \pm 3^{\circ}\text{C}$.



- 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