Title: **Routine Culture of iPSC**	Revision Number: 2	Effective Date: 27-Jun-2025
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1.0 PURPOSE

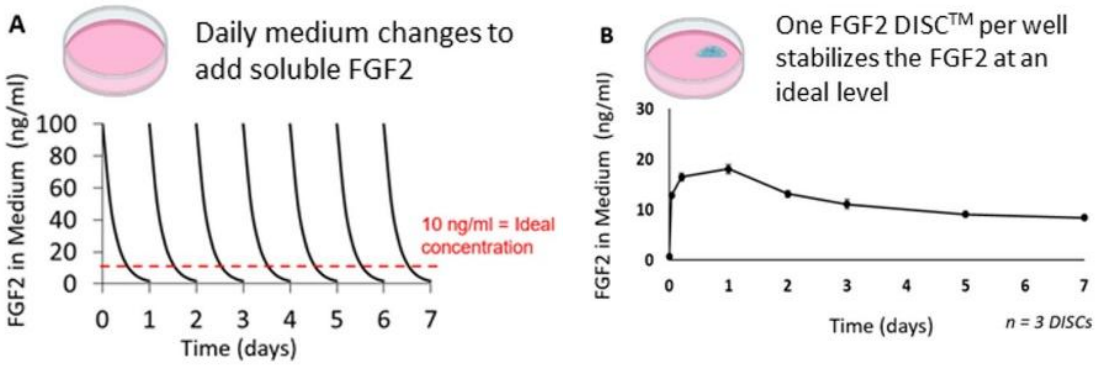
1.1. This SOP will discuss the procedures for passaging iPSC as colonies and subsequent maintenance of the iPSC culture.

2.0 SCOPE

2.1 All trained and approved members of the NeuraCell core facility will be responsible for compliance with this SOP.

3.0 DEFINITIONS

3.1 **iPSC:** Induced Pluripotent Stem Cells.
3.2 **MG:** Matrigel
3.3 **DMEM/F12:** Dulbecco's Modified Eagle's Medium (DMEM) and Ham's F12 with GlutaMax
3.4 **mTeSR:** Defined feeder-free maintenance medium for hESCs and hiPSC
3.5 **dPBS -/-:** DulBecco's Phosphate Buffered Saline without Calcium or Magnesium
3.6 **FGF2 DISC:** FGF2 DISCs stably release FGF2 over a period of 7 days



4.0 REFERENCES

4.1 Image supplement: page 5

5.0 SAFETY

5.1 Operators must follow all general lab safety practices. Gloves and a lab coat are required for this procedure.

6.0 MATERIALS AND EQUIPMENT

6.1 Gloves

6.2 Lab Coat

6.3 Sterile forceps

6.4 Bio-safety cabinet

6.5 37C incubator with 5% CO2

6.6 70% Ethanol

6.7 Matrigel (BD Biosciences, 354277) or Cultrex (BioTechne, 356230)

6.8 DMEM/F12 (Gibco, 11320033)

6.9 ReleSR (Stem Cell Technologies, 100-0483)

6.10 mTeSR1 (Stem Cell Technologies, 85850)

6.11 FGF2 DISCs (StemCultures, DSC500-48)

7.0 PROCEDURE FOR ROUTINE CULTURE

7.1 Prepare Matrigel/Cultrex coated plates according to manufacturer's instructions.

7.1.1 For iPSC expansion: Matrigel coated 6 well plates.

7.1.2 Incubate the Matrigel plate at 4°C at least overnight before using.

7.1.3 At least 20 minutes before use, incubate the Matrigel plate at 37°C

7.1.4 After warming the plate, add 2mL/well of mTeSR1

7.2 Passage and maintenance of iPSCs – 6-well plate:

7.2.1 Remove spent medium and DISC from iPSC well.

7.2.2 Rinse each well with 2mL dPBS -/-.

7.2.3 Remove dPBS -/-

7.2.4 Add 1.5ml of ReLeSR to each well being passaged.

7.2.5 Incubate plate at 37°C for 4 minutes.

7.2.6 Remove ReLeSR solution.

7.2.7 Dropwise, gently add 1mL/well of mTeSR1 to each well being passaged.

7.2.8 Use 1000uL micropipette to gently break the colonies into small pieces (usually only 1 or 2 triturations).

7.2.9 Dropwise, plate cell suspension at the desired ratio of iPSC colony pieces (usually 1:8-1:12) onto a new Matrigel-coated plate depending on lift success and desired growth rate.

7.2.10 Place plate in incubator (37°C with 5% CO_2) and gently shake back and forth, side to side to evenly distribute new colonies in the well.

7.2.11 After 24 hours, confirm cells have landed with a microscope, then exchange medium to remove any cell debris. Add one FGF2 DISC per well. See Image Supplement page 5.

7.2.12 Exchange medium 2-3 times a week with 2mL mTeSR1. Do Not remove FGF2 DISC. Slowly remove old medium, careful not to aspirate the

DISC. Add 2 mL of mTeSR1 to each well. Visual instructions for how to perform a medium change without removing the DISC can be seen at this link: https://stemcultures.com/discs/, video time 1:54 – 2:48. No weekend feeding necessary.

7.3 After ~5-7 days the colonies should be ready to be passaged again.

8.0 Image Supplement

Image of how to add FGF2 DISC using sterile forceps

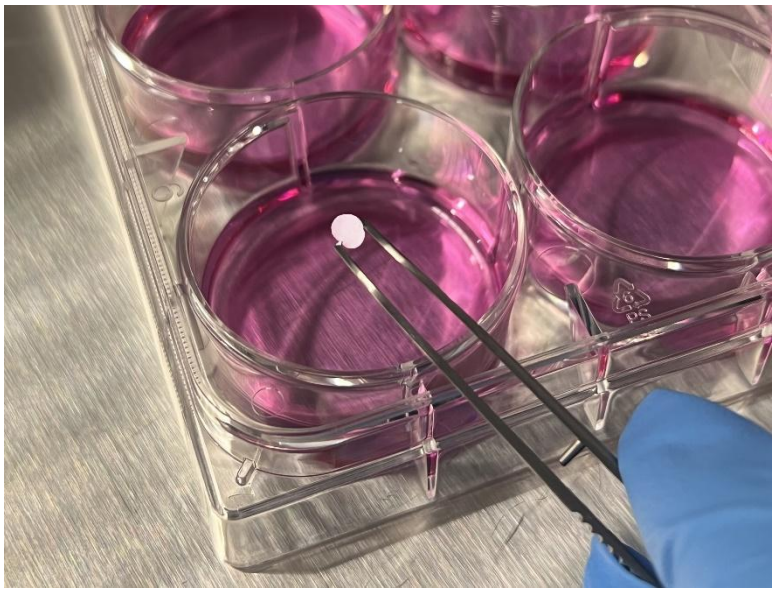
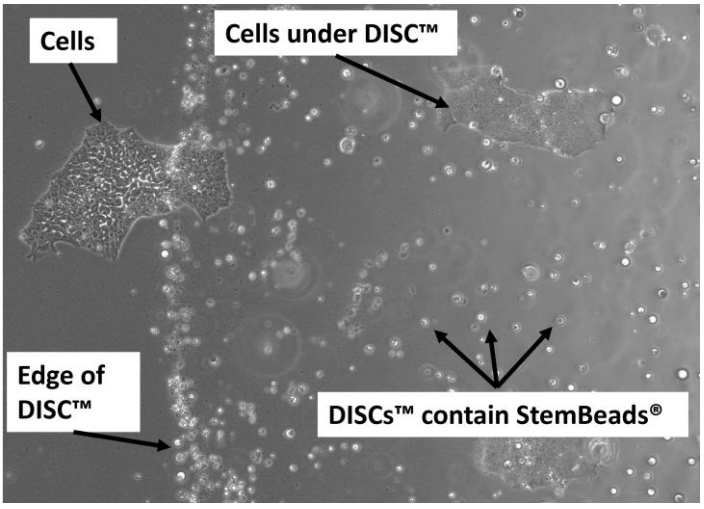


Image of an FGF2 DISC viewed under a microscope.



9.0 REVISION HISTORY

Revision Number	Effective Date	Description of Changes	Reason for Changes
1	November 13, 2024	New Document	N/A
2	June 27,2025	Minor edits	Missing information

APPROVAL SHEET

Signatures below indicate approval of the contents of this document.

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