StemCultures

**Training Guide**

Cell Culture Basics

Including Sustained Release Technology

# Overview

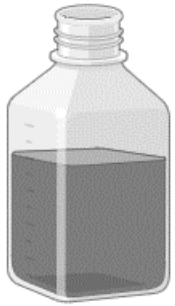
- Part 1: General Cell Culture
- Part 2: Cell Culture Using StemBeads®
- Part 3: Cell Culture Using DISCs™
- Part 4: Supporting Data

# PART 1: General Cell Culture

# Cell culture at a glance

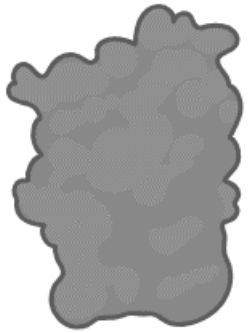
- Cell culture refers to aseptic laboratory methods that allow the growth of cells in artificial conditions.
- Applications in cellular biology, drug development, artificial meat production, research and development, etc.
- Note: each cell type requires different nutrients and feeding schedules
  - We focus primarily on induced pluripotent stem cell (iPSC) culture

# Raw materials universally needed for cell culture



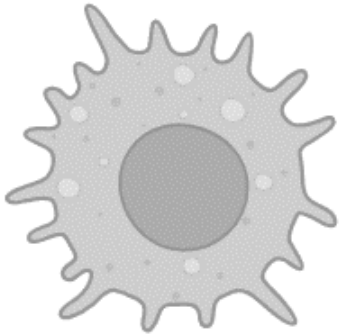
**Medium**

*Ex. DMEM/F12*



**Serum/Growth Factors**

*Ex. Fetal Bovine Serum (FBS) or FGF2*



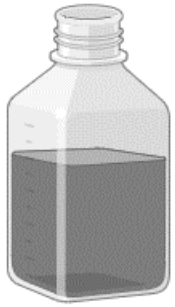
**Cells**

*Ex. iPSC, Neural Progenitor Cells (NPCs)*

**Culture Vessel**

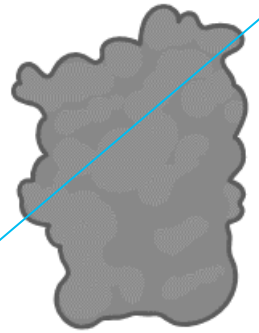
Ex. *cell culture plates (6 well vs 24 well, etc.), flasks, etc.*

# Raw materials universally needed for cell culture



**Medium**

*Ex. DMEM/F12*



**Serum/Growth Factors**

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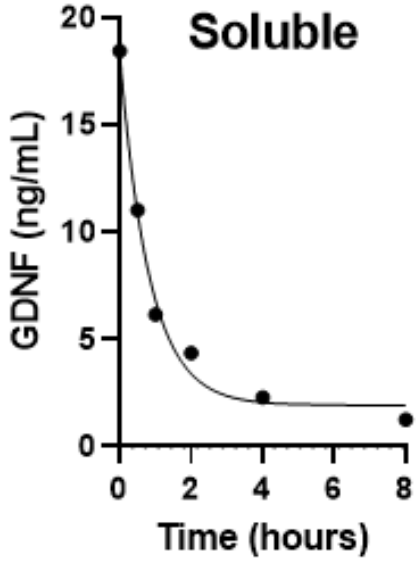
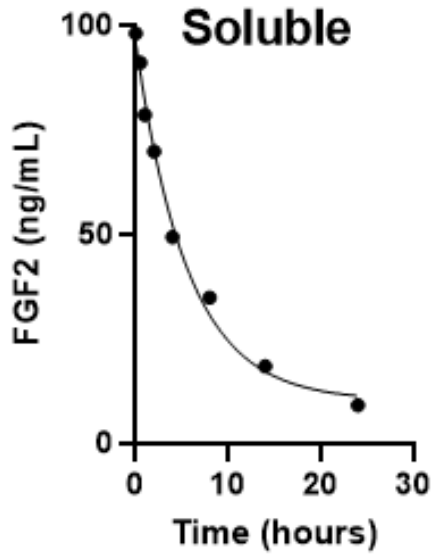



GDNF half-life

**0.5 hour**




FGF2 half-life

**4.5 hours**

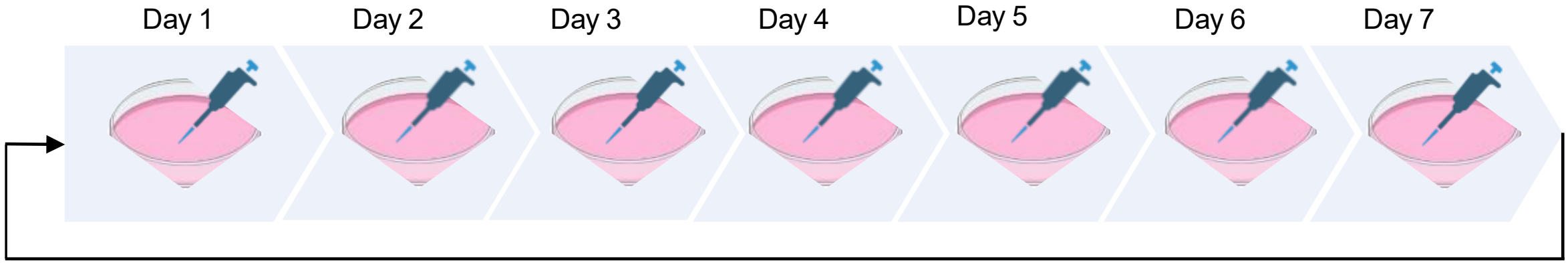
*(NPCs)*

*flasks, etc.*

# Main processes used in cell culture

- **Thaw** – the process of preparing a frozen vial of cells for further culture
- **Passage** – the process of harvesting cells from a culture, transferring them into a new vessel with fresh growth medium, and using those cells to start new cultures
- **Feed** – the process of changing/replacing culture media
- **Freeze** – the process of using low temperatures to preserve cells and tissues for future use

# Basic cell culture schedule (iPSC)




*Repeat every week*

| Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|
| Passage cells<br>Add medium | Feed cells | Feed cells | Feed cells | Feed cells | Feed cells | Feed cells |

# PART 2: Cell Culture Using StemBeads®

# What are StemBeads®?

- Inert, biodegradable, biocompatible microbeads
- Release sustained levels of native growth factors over several days
- Available for FGF2, EGF, BDNF, GDNF, and Activin A

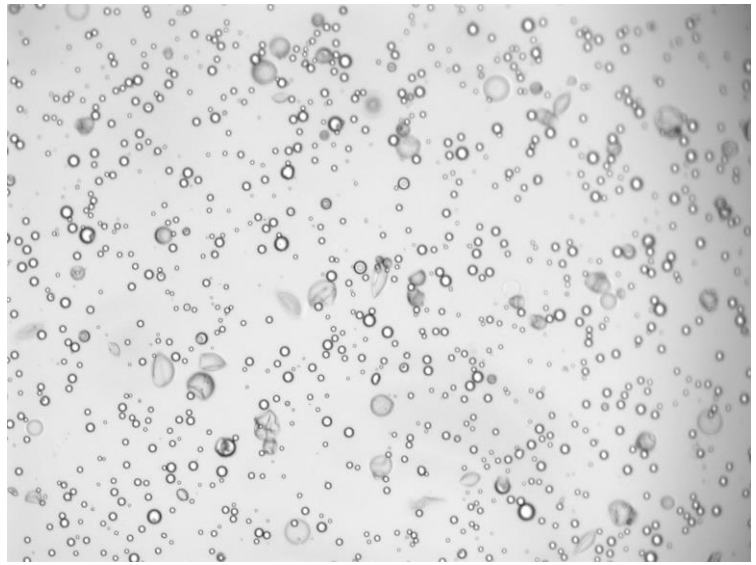
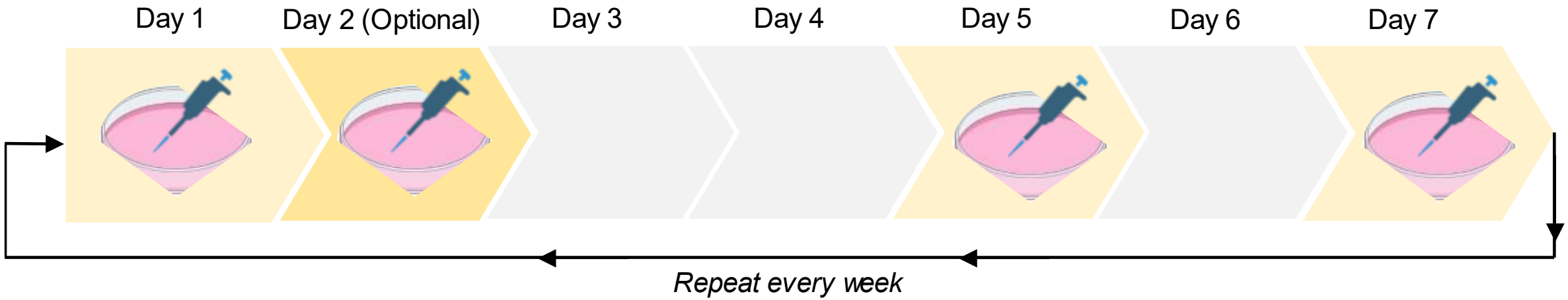


Figure 1: StemBeads® at 10x Objective

Average size: 10-20 µm Diameter

# Basic cell culture schedule (iPSC) with StemBeads®



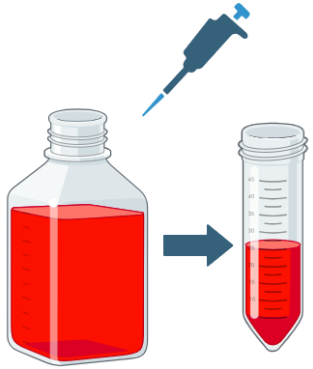

| Day 1 | Day 2 (Optional) | Day 5 | Day 7 |
|---|---|---|---|
| Passage cells<br>Add StemBeads® supplemented medium | Feed and wash cells<br>Replace StemBeads® medium | Feed and wash cells<br>Replace StemBeads® medium | Feed and wash cells<br>Replace StemBeads® medium |

Note: Different cell lines, cell types, and densities may require adjusted schedules. Please monitor your cultures frequently and adjust accordingly.

# Preparation of StemBeads® supplemented medium

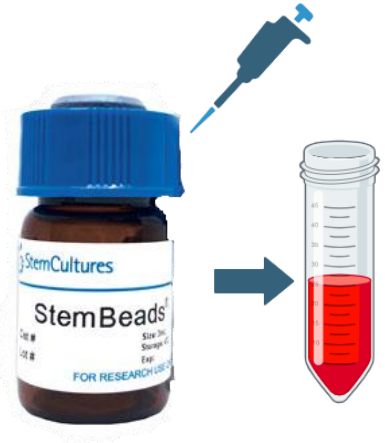

*Step 1*

Mix vial of StemBeads® thoroughly by vortexing or pipetting prior to use.



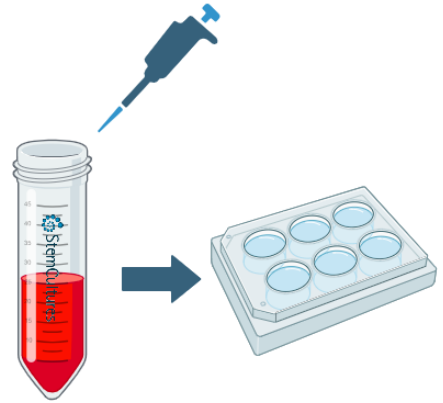
*Step 2*

Aliquot the necessary total volume of culture medium into a separate tube.




*Step 3*

Add StemBeads® to the medium aliquot to achieve the desired protein concentration.

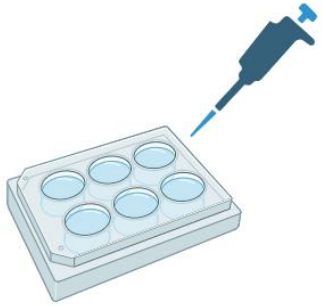


*Step 4*

Add the StemBeads® supplemented medium to your culture following your typical protocol.

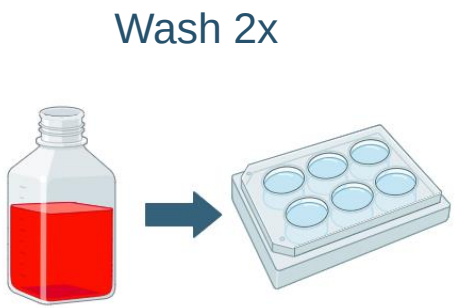
**Note:** when using sustained release products, the growth factor concentration can be reduced up to 10-fold compared to soluble.

# Washing StemBeads® for removal or feeding



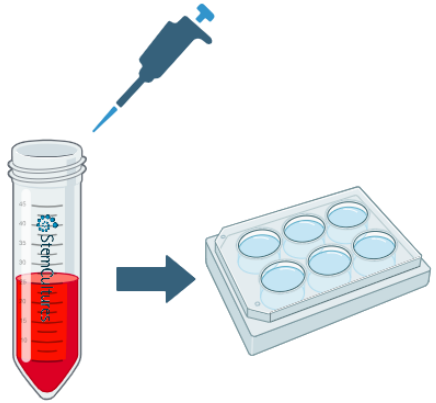
*Step 1*

Remove culture medium using a (vacuum) pipette.




*Step 2*

Wash cultures 2x with base medium such as DMEM/F12.



*Step 3*

Replace with StemBeads® supplemented complete medium.

**Note***:*

Washing is highly recommended prior to each feed to remove cell debris and remaining beads.

# PART 3: Cell Culture Using DISCs™

# What are DISC™ Devices?

- Inert, non-degradable, biocompatible hydrogel containing StemBeads®
- Release sustained levels of native growth factors into cell cultures over one to two weeks
- Easy to add and remove
- Available in FGF2, BDNF, and GDNF

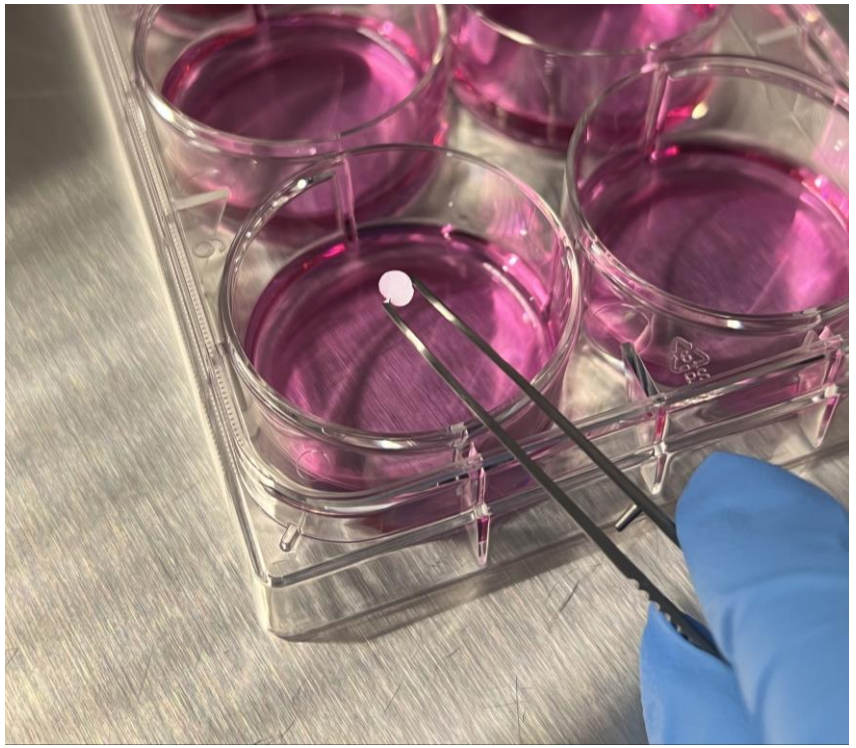
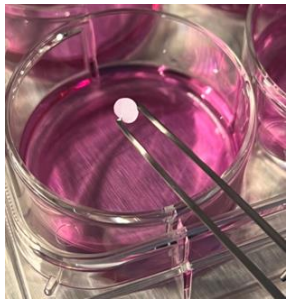
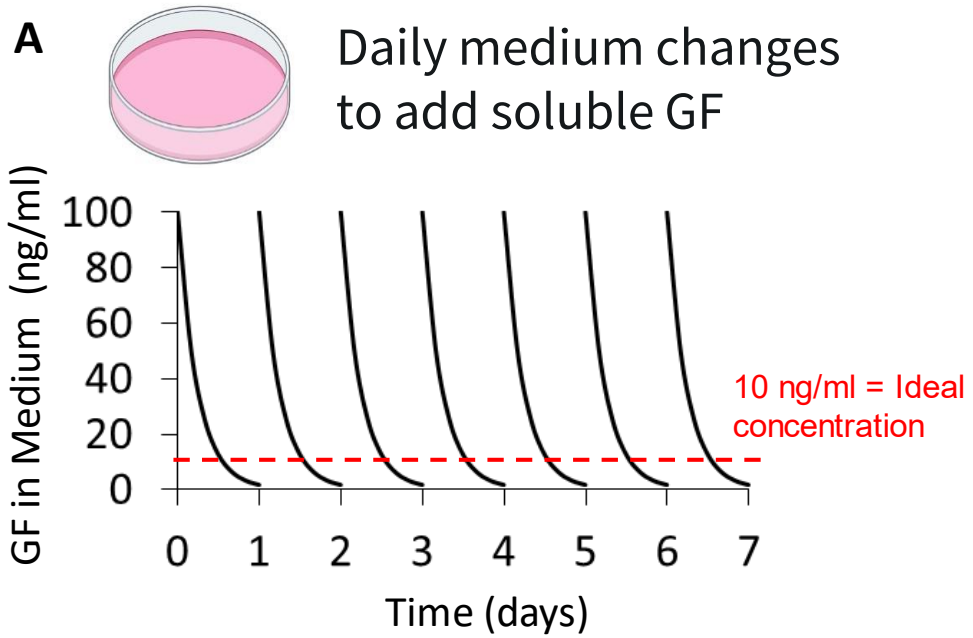


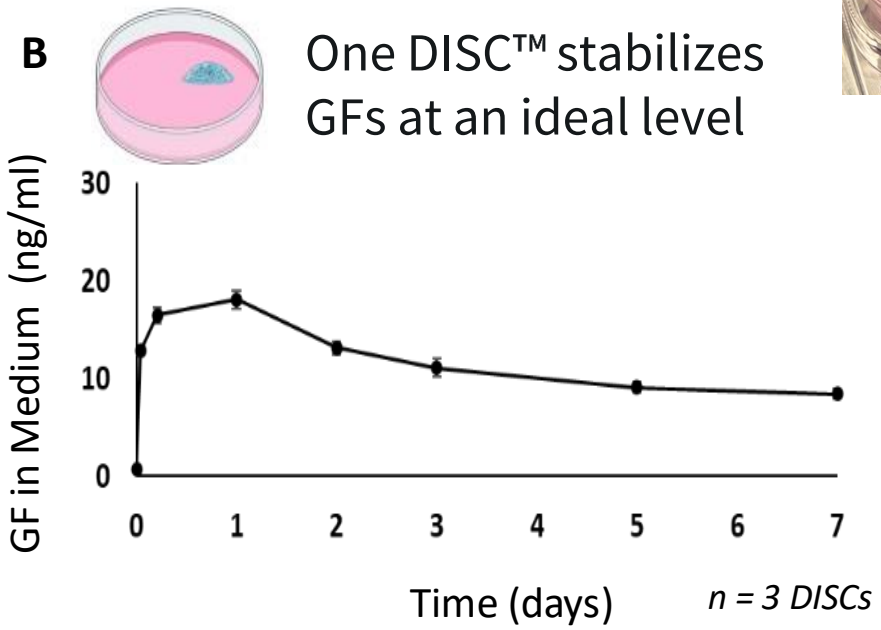
Figure 2: DISC™ Device being added into a culture well

Average size: 2-3mm diameter

# Controlled-release technology overcomes short protein half-lives in culture

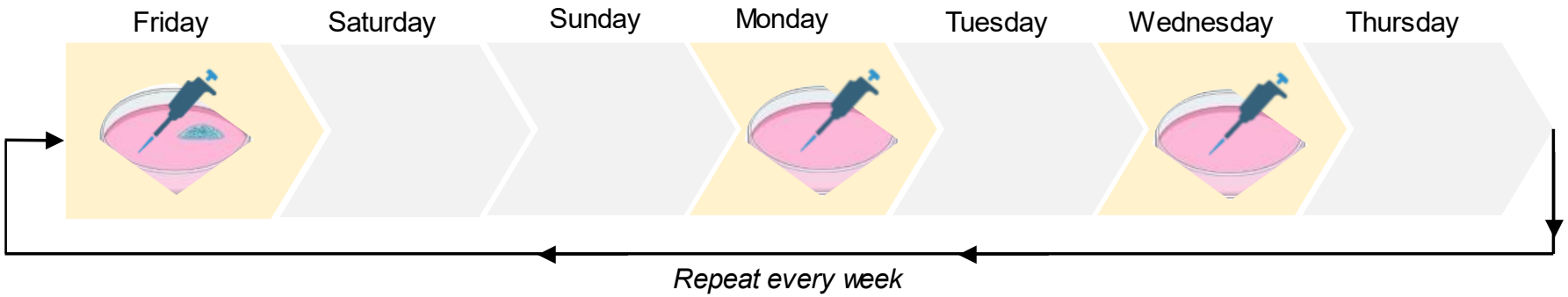





**Note:** when using sustained release products, the growth factor concentration can be reduced up to 10-fold compared to soluble

# Basic cell culture schedule (iPSC) with a DISC™




Passage cells

Add medium

**Add a DISC™**

Feed cells

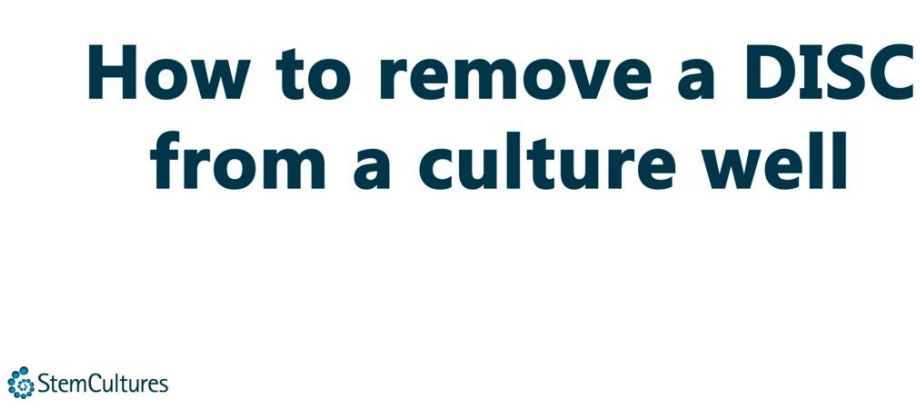
**Do not remove DISC™**

Feed cells

**Do not remove DISC™**

Note: Different cell lines, cell types, and densities may require adjusted schedules. Please monitor your cultures frequently and adjust accordingly.

# How to add and remove DISCs™ from a culture well


Full video link: https://youtu.be/vB1J-iCl2tw?si=6mdBx9Hh0HGoFV30

# How to feed cells when using a DISC™

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## How to perform a medium change with a DISC

StemCultures

Full video link: https://youtu.be/vB1J-iCl2tw?si=6mdBx9Hh0HGoFV30

# PART 4: Supporting Data

# Cell quality is improved with DISCs and reduced feeding as indicated by enhanced pluripotency and more efficient differentiations

Figure A

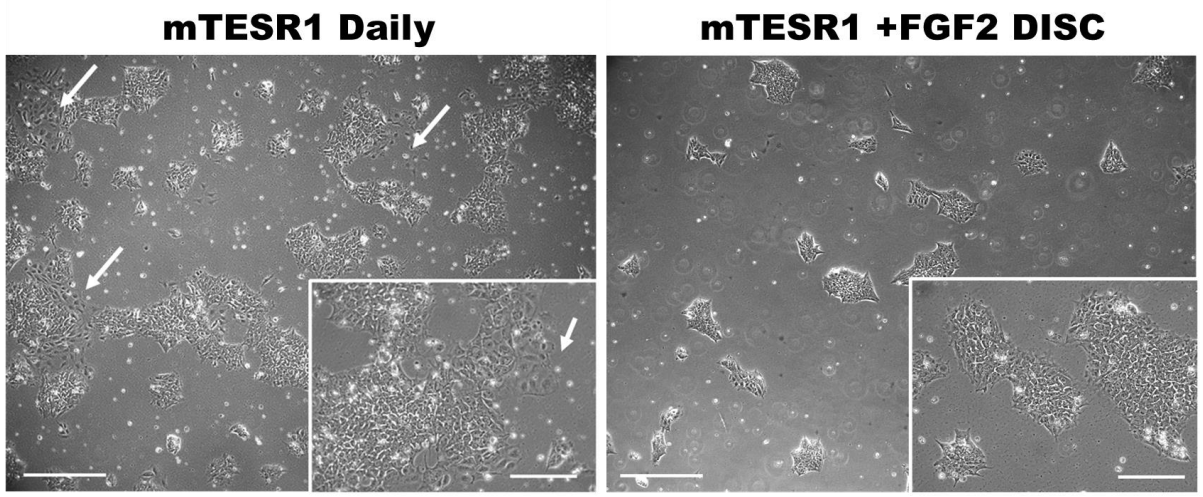
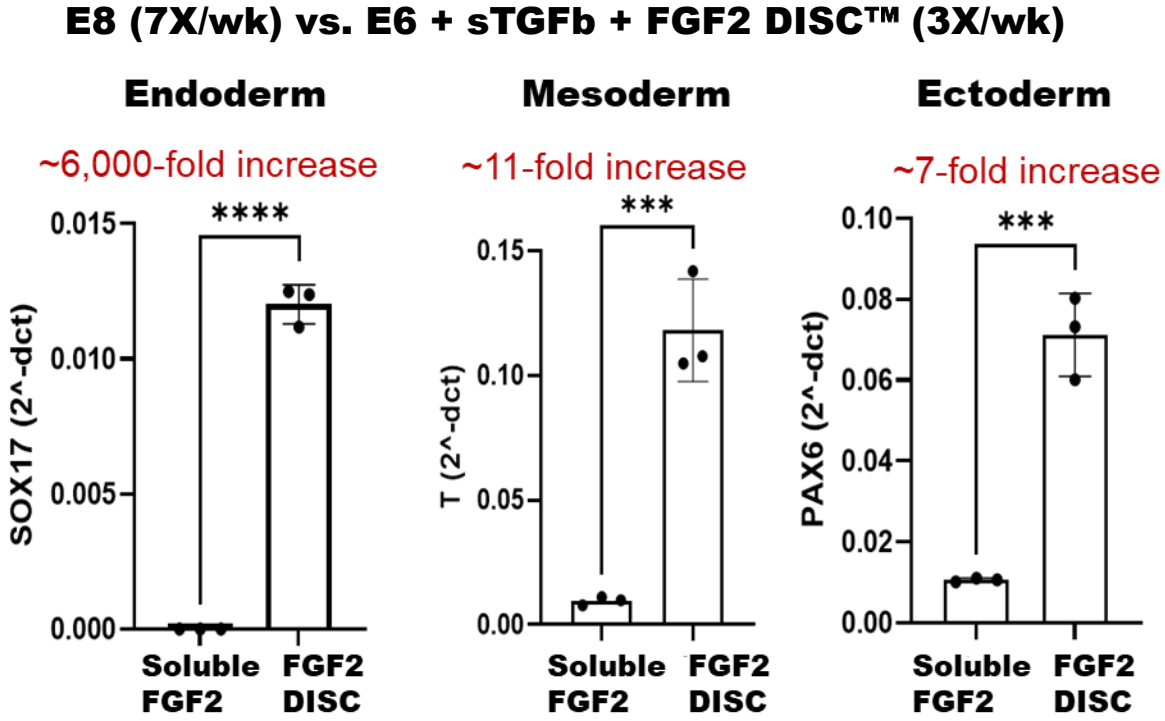



Figure B




Data generated in collaboration with NeuraCell CORE facility

# Downstream applications are enhanced with DISCs and reduced feeding

Figure A

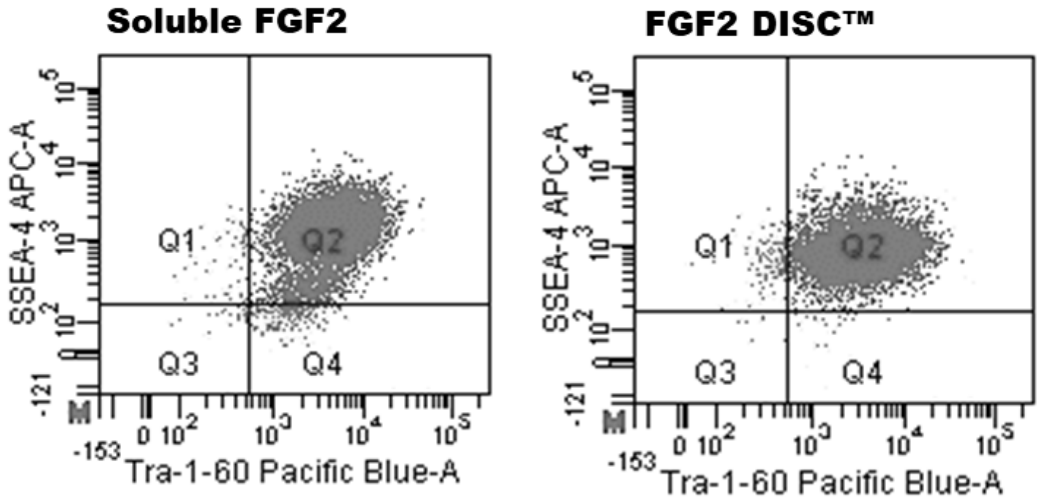
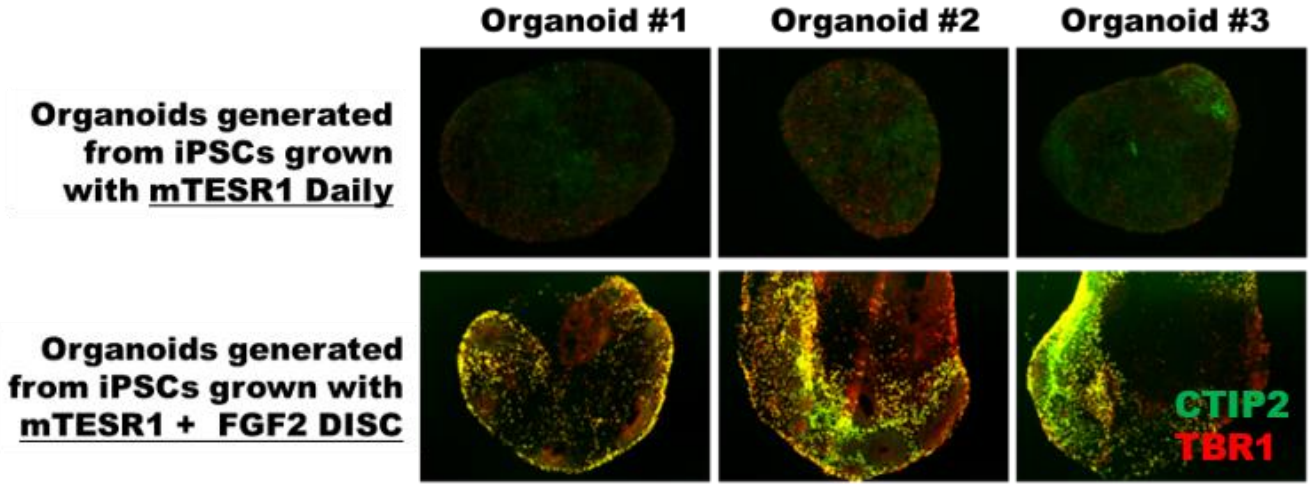



Figure B




Data generated in collaboration with NeuraCell CORE facility

# Publications that reference our products

- Direct differentiation of human pluripotent stem cells into vascular network along with supporting mural cells (Bertucci et al) – FGF2 DISCs
- Sustained levels of FGF2 maintain undifferentiated stem cell cultures with biweekly feeding (Lotz et al) – FGF2 StemBeads
- Cholesterol and matrisome pathways dysregulated in astrocytes and microglia (Julia TCW et al) – FGF2 StemBeads
- And more here: https://stemcultures.com/resources/publications/

StemCultures

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