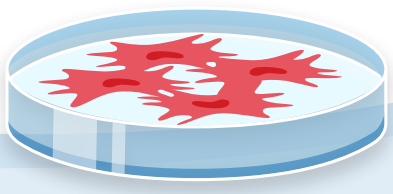
Stem Cell Therapy Production

Seeding, Expanding, and Harvesting Stem Cells

Key considerations for working with MSCs, iPSCs, and NSCs through different stages of the workflow



Mesenchymal Stem Cells (MSCs)

Growth as Individual Cells

Vessel selection

- Traditional: Dishes, plates, T-flasks
- Stacked vessels: Corning CellSTACK, and HYPERStack vessels
- Bioreactor: Corning Ascent Fixed Bed Reactor (FBR), microcarriers in bioreactor

Growth surface selection

- Surface treatment
  - TC-treated
  - Corning CellBIND
- Surface coating (pre-coated or self-coated)
  - Collagen
  - Fibronectin
  - Corning Synthemax II

Seeding Density

200-12,000 cells/cm^2 (most commonly 1,000-6,000 cells/cm^2)

Lower seeding density

- Increased proliferation potential/fold-expansion
- Fewer passages to reach target yield

Higher seeding density

- Reduced time to reach target cell density
- Economical for low output
- Increased stress to cells due to paracrine signaling, leading to stress fibers

Doubling Time

24-40 hours

Passaging Time

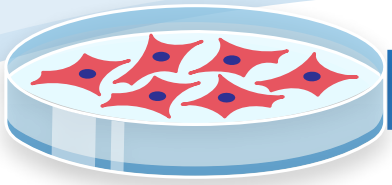
3-7 days

Target Confluency

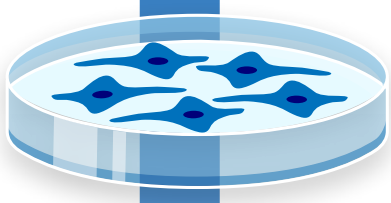
75-80%

Media Change

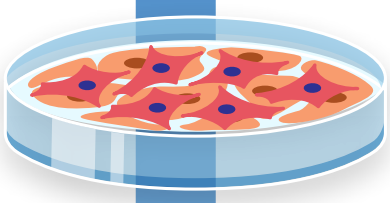
Every 2-3 days



Culture options and neural differentiation paths

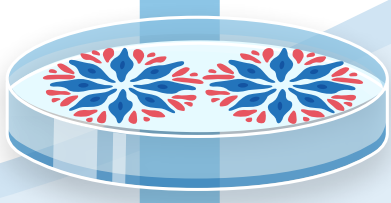


2D Monolayer

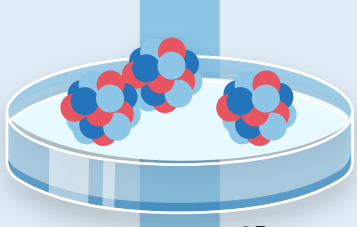


2D Coculture with stromal cells

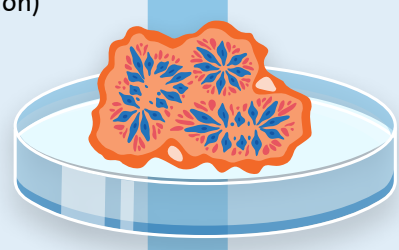
3D Embryoid bodies



2D Neural rosettes



3D Neurospheres



3D Brain organoids

Induced Pluripotent Stem Cells (iPSCs)

Growth as Clusters

Substrate on dishes, plates, T-flasks, or CellSTACK vessels

Mouse embryonic fibroblasts (MEFs)

- Irradiated animal cells
- Safety concerns

Corning Matrigel matrix

- From mouse sarcoma cells
- Not fully defined

Corning Synthemax II vitronectin substrate

- Synthetic
- Xeno-free

Corning rLaminin-521 (human)

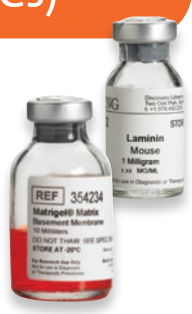
- Recombinant human Laminin protein

Growth as Individual Cells

Recommend with Ascent FBR or microcarriers and bioreactor

Seeding Density

10,000-20,000 cells/cm^2



Doubling Time

16-20 hours

Passaging Time

4-5 days

Passaging Criteria

60-75% confluency and/or medium-to-large colony size and/or signs of spontaneous differentiation

Microscopic observation daily for

- iPSC-like morphology
- Differentiated cells
- Confluency

Accutase cell detachment solution

- Gentle, enzyme-free dissociation preserves genomic stability

Corning CellStripper solution

- Non-enzymatic cell dissociation solution formulated with a proprietary mixture of chelators

Manual passaging with pipet tip or cell scraper

Media change

Daily

Neural Stem Cells (NSCs)

Growth at High Density

Growth surface to promote attachment on dishes, plates, T-flasks, or CellSTACK vessels

- TC-treated
- CellBIND

Add positive charge

- Poly-L-Ornithine
- Poly-L-Lysine
- Poly-D-Lysine
- Corning PureCoat Amine

Add Extracellular Matrix (ECM)

- Laminin

Seeding Density

20,000-100,000 cells/cm^2

Doubling Time

20-48 hours (very limited before differentiation)

Confluency at Passaging

95-100%

Learn more about the most important considerations for working with different stem cell types — MSCs, iPSCs and NSCs — through different stages of the workflow.

www.corning.com/celltherapy

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