



Working with patient-derived intestinal/colonic fibroblasts

Last updated: 11/10/2023 (TK)

Reagents:

- Nutragen Bovine Collagen Solution, Type I (Advanced Biomatrix 50360232 - 5010-D)
 - *Other sources of type I collagen are very likely to work but haven't been tested.*
- Dulbecco's phosphate buffered saline (D-PBS)
- Advanced DMEM/F12 1X (Thermo Fisher #12634-010)
- Fetal bovine serum (FBS): preferably HyClone
- Antibiotic-Antimycotic 100x (Thermo Fisher #15240062)
- Complete Primary fibroblast medium (1ry FbM):
 - Advanced DMEM/F12
 - 15% FBS
 - 1x Antibiotic-Antimycotic
- Trypsin-EDTA 0.05%



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Protocol:

1. Pre-coat the TC dish with collagen
 1. Cover the surface with collagen diluted 1:100 in D-PBS
 - *E.g., use 2 mL to coat a 60mm dish*
 2. 37°C - 30 min (e.g. incubator)
 3. Rinse the dish 3 times with D-PBS
2. Recover the cells into a dish size recommended on cryovial
 1. Quickly thaw the cryovial in a 37°C water bath
 2. Transfer the contents into a 1.5 mL tube
 3. Spin and remove the cryopreservation medium
 4. Wash with 1 mL D-PBS
 5. Reconstitute in 1ry FbM and transfer to the coated dish
3. Monitor growth, and passage only after reaching complete confluence (see the representative images below)
 - *Passage at low confluency may result in premature senescence*
 - *Recommended split rate 1:2 – 1:3, do not exceed 1:5*

Sparse culture

Confluent culture

