Madeline Lancaster

Cerebral organoids Version 2.0

**Materials**

* DMEM/F12: Invitrogen cat#11330-032
* KOSR: Invitrogen cat# 10828-028
* GlutaMAX: Invitrogen cat#35050-038
* P/S: Penicillin/Streptomycin: Sigma cat#P0781
* MEM-NEAA: MEM-Non-essential amino acids: Sigma cat#M7145
* 2-Mercaptoethanol
* bFGF (FGF2): Peprotech cat#100-18B
  + 10ug/ml solution prepared by reconstituting 50ug in 5ml PBS +0.1% BSA
  + Aliquot and store at -20C for up to 1 year
* 0.05% Trypsin/EDTA solution: Invitrogen
* Trypsin inhibitor: Sigma cat# T6414-100ML
* Heparin: Sigma cat#H3149
  + 1mg/ml solution in PBS – store at -4C
* Dispase: Invitrogen cat#17105-0412q
* Rock inhibitor Y27632, 5mg: VWR cat# 688000-5
  + Working solution prepared by reconstituting 5mg in 2.96 ml water
* N2 supplement: Invitrogen cat# 17502048
  + Aliquot and store at -20C for up to 1 year
* B27-vit. A supplement: Invitrogen cat# 12587010
  + Aliquot and store at -20C for up to 1 year
* B27+vit. A supplement : Invitrogen cat# 17504044
  + Aliquot and store at -20C for up to 1 year
* Neurobasal medium: Invitrogen cat# 21103049
* Sterile D-PBS w/o Ca and Mg
* Insulin solution: Sigma cat# I9278-5ML
* Spinner flask, 125ml size: Corning cat# 4500-125
* Horse serum: Invitrogen cat# 16050122
* HBSS, calcium, magnesium, no phenol red: Invitrogen cat# 14025092
* MEM, Glutamax, HEPES: Invitrogen cat# 42360024
* Vitamin C solution: Dissolve 352 mg Ascorbic Acid in 50 ml DMEM/F12, Store in dark at 4C

**Media**

Low bFGF hES media (500 ml) – Store at 4C up to 1 month

* 400 ml DMEM/F12
* 100 ml KOSR
* 15 ml ES-quality FBS
* 5 ml GlutaMAX
* 5 ml MEM-NEAA
* 3.5 ul 2-Mercaptoethanol
* Filtered using 0.22 um filter
* 4 ng/ml bFGF (Add just before use)

Dispase solution (10 ml) – Store at -20C up to 6 months

* 5 mg Dispase
* 5 ml DMEM/F-12
* Filtered using 0.22 um filter

Neural induction media (50 ml) – Store at 4C for up to 1 month

* 50 ml DMEM/F12
* 0.5 ml N2 supplement
* 0.5 ml Glutamax supplement
* 0.5 ml MEM-NEAA
* 50 ul Heparin solution
* Filtered using 0.22 um filter

Improved NeuroDMEM -A (250 ml) – Store at 4C for up to 1 month

* 125 ml DMEM/F12
* 125 ml Neurobasal
* 1.25 ml N2 supplement
* 5 ml B27- vitamin A supplement
* 62.5 ul insulin
* 87.5 ul 2-ME solution (1:100 dilution in DMEM/F12)
* 2.5 ml Glutamax supplement
* 1.25 ml MEM-NEAA
* 2.5 ml P/S

Improved NeuroDMEM +A (500 ml) – Store at 4C for up to 1 month

* 250 ml DMEM/F12
* 250 ml Neurobasal
* 2.5 ml N2 supplement
* 10 ml B27+ vitamin A supplement
* 125 ul insulin
* 175 ul 2-ME solution (1:100 dilution in DMEM/F12)
* 5 ml Glutamax supplement
* 2.5 ml MEM-NEAA
* 5 ml P/S
* 1.49g HEPES
* 5 ml Vitamin C solution (40mM stock)

Growth media + MG (50 ml) – Store at 4C for up to 2 weeks

* 50 ml Improved NeuroDMEM +A– Keep cold!
* 1 ml Matrigel – Slowly thaw on ice and add slowly to cold media to dissolve

Procedure

Follow protocol as in original method with the following timing modifications:

