

## MEI Protocol

### Oli-neu cell cultures

The cells are to be seeded in culture flasks coated with 0.01 % PLL

They need Sato-Media + 1% Horse Serum.

For passaging the cells use Trypsin-EDTA (TE low).

Always seed two to three different concentrations of cells because their growing character changes from time to time.

Good cell densities are: 0,5 - 2x10<sup>6</sup> cells/15 cm-dish

### PLL

10x-Stock: dissolve 1 g in 1 l sterile H<sub>2</sub>O, store in aliquots at -20° C

Working Solution: dilute 1:10 in Sterile H<sub>2</sub>O

Poly-L-Lysine Hydro bromide Degree of Polymerisation 450000

Sigma, #P-1524, 1 g

### Sato + 1% HS

	Stored at	100 ml	500 ml	1 l
DMEM-Powder	4° C	1.34 g	6.7 g	13.4 g
NaHCO <sub>3</sub>	RT	0.2 g	1 g	2 g
Transferrin	4° C	0.001 g	0.005 g	0.01 g
Dissolve in H <sub>2</sub> O <sub>bidest</sub>		95 ml	480 ml	950 ml
Insulin-Stock	-20° C	1 ml	5 ml	10 ml
Putrescine	-20° C	1 ml	5 ml	10 ml
Progesteron	-20° C	10 µl	50 µl	100 µl
TIT	-20° C	100 µl	500 µl	1 ml
Sodium-Selenit	4° C	74 µl	370 µl	740 µl
L-Thyroxin	4° C	13 µl	65 µl	130 µl
Adjust with H <sub>2</sub> O <sub>bidest</sub> to		100 ml	500 ml	1 l
Gentamicin	4° C	50 µl	250 µl	500 µl
Sterile filtrate				
Horse Serum	4° C/ -20° C	1 ml	5 ml	10 ml

DMEM-Powder                      Invitrogen #52100-039

Apo-Transferrin human        Sigma #T-2252

Insulin                                Sigma #I-5500 (-20°C)  
Stock: 0.01g in 10ml H<sub>2</sub>O + 20µl 1N HCL, stored aliquoted at  
-20°C

Putrescine	Sigma #P-7505 (RT) 161.1g/mol Stock: 0.0161g in 10ml H <sub>2</sub> O = 10mM stored aliquoted at -20°C
Progesterone	Sigma #P-0130 (RT) Stock: 0.62 mg / ml 96% ETOH (store at -20°C)
Tri-Iodo-Thyrodine	Sigma #T6397 (-20° C) 0.0034g in 10ml 96% ETOH (store at -20°C)
Sodiumselenit	Sigma #S1382 (RT) 300µM Selenium: Stock 1: 0.05187g / 10ml H <sub>2</sub> O = 30µM Stock 2: 100µl Stock 1 + 9.9ml H <sub>2</sub> O = 300µM (4°C) (MG=172.9g/1000ml = 1M = 1000mM 0.05187g/1000ml = 0.3mM = 300µM)
L-Thyroxine	Sigma # 89430 (4°C) Na-Salt in 0.13N NaOH in 70% ETOH, = 0.062g in (0.104g NaOH in 6ml H <sub>2</sub> O + 14ml ETOH) (4°C)
Gentamicin	Invitrogen # 15750-037 50mg/ml Medium

### **TE (Trypsin-EDTA) low**

0.2 ml 1% Trypsin  
2 ml 0.2% EDTA in HBSS (Invitrogen #14170-138)  
Adjust to 20 ml with HBSS  
Sterile filter  
At 4° C stable for 1 week

### **1% Trypsin**

100 ml 2.5 % Trypsin (Invitrogen #15090-046)  
15 ml HBSS 10x  
135 ml H<sub>2</sub>O  
125 mg DNase I (Roche, 104159, 100 mg)  
Adjust pH=7.8 with 2 N NaOH  
Sterile filter  
Aliquots stored at -20° C

### **PBS/10 % HS**

To stop trypsinisation use cold 1x PBS (or DMEM) with 10 % Horse serum