

Preparation of 2i medium

DMEM/F-12, GlutaMAX™ Suppl. (Life 31331-028)	250 ml
1X Neurobasal® Medium (Life 21103-049)	250 ml
50X B-27® Serum-Free Suppl. (Life 17504-044)	5 ml
100X N-2 Suppl (Life, 17502-048)	2,5 ml
BSA 25mg/ml (Sigma, A3311-10G)	0,5 ml
GlutaMAX™ Suppl. (35050-038)	2,5 ml
Insulin 20mg/ml (Sigma, I1882-100MG)	0,25 ml
Pen/Strep (Life, 15140-122, stock)	5 ml
FCS ~2%	10 ml
<i>1-Thioglycerol (Sigma, M6145-25ML)</i>	<i>14 µl</i>
<i>PD 0325901 10mM (Axon, 1408)</i>	<i>50 µl</i>
<i>CHIR 99021 10mM (Axon 1386)</i>	<i>150 µl</i>
<i>Lif (EMBL)</i>	<i>15 µl</i>

Neuronal differentiation medium

DMEM/F-12, GlutaMAX™ Suppl. (Life 31331-028)	250 ml
Neurobasal®-A Medium (Life, 10888-022)	250 ml
B-27® Supplement (50X), minus vit. A (Life, 12587-010)	5 ml
100X N-2 Suppl (Life, 17502-048)	2,5 ml
BSA 25mg/ml (Sigma, A3311-10G)	0,5 ml
GlutaMAX™ Suppl. (35050-038)	2,5 ml
Insulin 20mg/ml (Sigma, I1882-100MG)	0,25 ml
1-Thioglycerol (Sigma, M6145-25ML)	7 µl
Heparin 50 mg/ml (Sigma, H3149-10KU)	10 µl
Pen strep (Life, 15140-122, stock)	5ml

Fresh every day:

FGF-basic, 0,5 mg/ml (Preprotech, 450-33)	20000x
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Differentiation protocol:

Plate on **laminin** (diluted 1/1000 in gelatin, incubated 2h at 37°C. Plate **0,8x10⁶ cells / 90mm dish**, **two 90mmsishes by day 3 of differentiation are enough to obtain 15 – 40 µg of RNA. ~2,5 mil NPCs at day 3. Change medium every day.**

PD 0325901 (10mM): Dissolve 5mg in 1030µl of DMSO. Aliquot in 50µl portions.

CHIR 99021 (10mM): Dissolve 5mg in 1070µl of DMSO. Warming might be required to dissolve. Aliquot in 150µl portions.

Insulin (20mg/ml): Dissolve 100mg of Insulin in acidified water (100µl of glacial acetic acid to 10ml of water.

FGF-basic (0,5 mg/ml): Dissolve 50µg in 100µl water. Aliquot in 10µl portions.