|  |  |  |
| --- | --- | --- |
| **NSPC maintenance Medium**  **(NMM)** | **NSPC induction medium**  **(NIM)** | **NSPC starvation medium** |
| Neural Basal Medium (50%) | Neural Basal Medium (50%) | Neural Basal Medium (50%) |
| DMEM/F-12, GlutaMAX (50%) | DMEM/F-12, GlutaMAX (50%) | DMEM/F-12, GlutaMAX (50%) |
| B27 supplement w/o Vit A (1X) | B27 supplement w/o Vit A (1X) | B27 supplement w/o Vit A (1X) |
| N2 Supplement-B (1X) | N2 Supplement-B (1X) | N2 Supplement-B (1X) |
| P/S (1X) | P/S (1X) | P/S (1X) |
| FGF2 (10 ng/ml) | FGF2 : 5 ng/ml |  |
| EGF (10 ng/ml) | LDN193189 500 nM |  |
| BDNF (20 ng/ml) | SB431542 20 µM |  |
|  | +/- Y-27632 10 µM |  |

Table 1: Recipe of the media used for the generation of 2D-neural rosettes and NSPC

**EB medium Induction medium-1 Induction medium-2**

DMEM-F12/Glutamax DMEM-F12/Glutamax DMEM-F12/Glutamax

20% (v/v) KoSR 20% (v/v) KoSR 1x N2

1× MEM-NEAA 1× MEM-NEAA 1× MEM-NEAA

100 µM b-ME (55mM) 100 µM b-ME (55mM)  5 µM SB431542

P/S 0,1% P/S 0,1% P/S 0,1%

Y-27632 50µM 200 nM LDN-193189/Dorso 5µM

+/- bFGF 4ng/ml  5 µM SB431542

**Differentiation medium-1 Differentiation medium-2**

DMEM-F12/ Glutamax DMEM-F12/ Glutamax

1x MEM NEAA 1x MEM NEAA

1x B27 w/o Vit A1x B27 with Vit A

1x N2 1x N2

50 µM b-ME (55mM) 50 µM b-ME (55mM)

Insulin 2,5µg/ml Insulin 2,5µg/ml

P/S 0,5% P/S 0,5%

Matrigel 1% (from Day 35)

Supplemental table 2: Recipe of the media used for the generation of dorsal forebrain organoids