Primary Neurosphere Culture

Materials

MHM with p/s (MHM)

name	dose	ratio
MHM	500 mL	-
p/s	500 uL	1:1000

NS medium

name	dose	ratio
MHM with p/s	50 mL	-
B27	1000 uL	1:50
FGF	50 uL	1:1000
EGF	50 uL	1:1000

Methods

Culture (from PNS stock)

- defrost PNS stock in 37°C water bath
- PBS wash x1
  - centrifuge: 1200 rpm x5min
  - aspiration
- resuspend with NS medium 40mL, apply all to Ultra-low attachment T75
- after confirmation with microscope, transfer to incubator
- culture for 5 days (approx.)

Immunofluorescense

day1

- prepare `adherent 24-well dish for observation`
- aspirate fibro. in the wells
- apply NS medium 500uL each
- transfer culturing suspension 200uL each from T75 into wells
- 37°C incubation for 30-40 min

- confirm with microscope that nerospheres firmly adhere to the floor
- aspiration
- process with PFA x45min (approx.) in RT
- PBS wash x4
- process with 0.3% TX x15min (approx.) in RT
- PBS wash x2
- apply Primary Ab Mix
  - e.g.) Primary Ab Mix (for 1 well)

name	dose	ratio
Blocking One	500 uL	-
anti-xxx Mouse mAb	0.5 uL	1:1000
anti-yyy Rabbit mAb	0.5 uL	1:1000

- 4°C o/n with shading

day2

- PBS wash x3
- apply Secondary Ab Mix
  - e.g.) Secondary Ab Mix (for 1 well)

name	dose	ratio
Blocking One	500 uL	-
anti-Mouse Gt	0.5 uL	1:1000
anti-Rabbit Dk	0.5 uL	1:1000

- shade in RT x1hr (approx.)
- PBS wash x2
- apply 1:1000 hochest

name	dose	ratio
PBS	500 uL	-
hochest	0.5 uL	1:1000

- shade in RT x5min (approx.)

- PBS wash x2
- wait until wells get dry
- observe with KEYENCE