293T Cell Culture

Materials

293T medium

name	dose	ratio
DMEM	500 mL	-
FBS	50 mL	1:10
p/s	5 mL	1:100

Methods

Culture (from stock)

- defrost 293T cell stock in 37°C water bath
- PBS wash x1
  - centrifuge 1200 rpm x5min
  - aspiration
- ressuspend with 10 mL 293T medium and apply into T25
- after confirmation with a microscope, transfer to the incubator
- culture for 5 days (approx.)

Passage

- pipetting for several times to dettach cells from T25
- apply all to 15mL tube
- centrifuge 1200rpm x5min
- aspiration
- resuspend with 40mL 293T medium
- apply all to T75
- after confirmation with a microscope, transfer to the incubator
- culture for 5 days (approx.)

Immunofluorescense

day1

- prepare `adherent 24-well dish for observation`
- aspirate fibro. in the wells
- apply 293T medium 500uL each
- after pipetting for several times, transfer culturing suspension 200uL each from T25 into wells
- 37°C incubation o/n

day2

- confirm with microscope that 293T 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

day3

- 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