

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
- resuspend with 10 mL 293T medium and apply into T25
- after confirmation with a microscope, transfer to the incubator
- culture for 5 days (approx.)

Immunofluorescence

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 for 30-40 min
- 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 |

| name | dose | ratio |
|---------------------|--------|--------|
| 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