TechComm
科技共同空間

Cell Fusion

Before fusion:

- **3~5** days before fusion, boost without Adjuvant.
- Prepare a T75 SP2 in mid-log phase, 1 day before fusion, change medium and reduce half cell. At fusion day, cell number ~**10^7** (30~50 $\times 10^4$/ mL)
- **1 week** before fusion, check reagents and medium, **3 days** before fusion, check surgical instrument, **1 day** before fusion, turn on UV overnight.

Harvest spleen

Warm DMEM & 0.5 L H_2O | Warm DMEMX+HAT

- Sacrifice mouse by CO_2 (1~3 min)
- Wet the fur with 75% EtOH
- Move to Laminar flow, fix mouse on dissection board
- Open thoracic & bleed from the heart
- Cut the whole spleen
  Rinse the spleen in 2 Petri dishes filled with DMEM
- Stab the spleen, wash with DMEM & Press the spleen throw a cell strainer
- **Centrifuge** (300 rcf, 10 min)
  Decant supernatant and disruptpellet.
- Lyse RBC & **Centrifuge** (300 rcf, 10 min)
  Decant supernatant and disruptpellet. Add DMEM to 50 mL

Cell Fusion

- Mix
- **Centrifuge** (300 rcf,10 min)
  Warm water & fusion instrument standby
- Decant supernatant and disrupt pellet

Golden Six min

- **1** min add 0.7 mL PEG (shaking the tube)
- **1** min Shake hard
- **2** min add 2 mL DMEM (shake slow down)
- **2** min add 8 mL DMEM

- **Centrifuge** (200 rcf, 8 min)
- Decant supernatant and disrupt pellet, add 45 mL DMEMX-HAT
- Distribute to three 96-well plates (add 150 μL/well, incubate in 37℃ Incubator)

FDay 0 ⇨ *Screening*

Wash Cells

Drop 12 μL, count spleen cells(5/6)

- Transfer splenocytes suspension to a new tube through the cell strainer (100 μm)
- **Centrifuge** (300 rcf,10 min)
- Decant supernatant, disrupt the pellet.

Clean instruments Pack mouse body

At the same time — Warm PEG

Transfer SP2 to a new sterile tube. Add DMEM to 50 mL

Drop 12 μL, count SP2 cells(1/6)

- **Centrifuge** (300 rcf,10 min)
- Decant supernatat, Disrupt the pellet. Add DMEM to 50 mL

3:1 ~ 10:1

Mix ⇨ Cell Fusion

Instruments list :

50 mL tube	x4
Styrofoamboard	x1
Surgical instrument	x2
Pin	x5
Timer	x1
Syringe	x2
96 Well Plate	x3
Petri dishes	x2
500 mL beaker	x1

Reagents list :

DMEM	250 mL
DMEMX+HAT (15% FBS)	45 mL
PEG	0.7 mL