

# TC4-SOP 0-7 Cryopreservation of Cells

Myeloma  Hybridoma Project Name: \_\_\_\_\_

## MATERIALS:

- Freezing medium : 4 mL FBS + 3 mL DMEM + 1 mL DMSO or **10% DMSO with FBS**  
 Fetal Bovine Serum (FBS) :  
 DOLBECCO'S MEM (DMEM) :  
 Dimethyl Sulfoxide (DMSO) :
- Cryovials, sterile
- Plastic dropper, sterile
- 50 mL conical tube
- Ice bucket
- Freezer storage box (Styrofoam)
- Centrifuge
- - 80°C freezer
- Laminar flow
- Piptmen P1000

## METHODS:

- Grow cell line to be frozen to mid-log phase in a T25/T75 culture flask.
  - Change medium \_\_\_\_ / \_\_\_\_ / \_\_\_\_ :
- 30 min before the operation :
  - Mix Freezing medium:  
 Add **3 mL DMEM** to a 50 mL tube in ice water, then add **1 mL DMSO**, final add **4 mL FBS**.
  - Label sterile cryovials with name of cell line, freeze date, and operator.
  - Wipe the culture hood with 75 % ethanol, then turn on the UV light until the operation.
- Use a sterile plastic dropper or pipet to flush the myeloma/hybridoma cells.
- Transfer the cells to a sterile 50 ml conical centrifuge tube. Centrifuge the cells 5 min at 300 rcf, room temperature. Discard the supernatant and save the pellet.
- Resuspend the pellet gently to disperse the cells.
- Add 1 mL freezing medium in 1 min, then add freezing medium to 3 mL (Total volume depend on the hybridomas cell number\*).
- Transfer 1 ml of resuspended cells to each labeled cryovial.
- Cap the vial and place in a freezer storage box in a **-80°C** freezer a.s.a.p.
- \_\_\_\_ / \_\_\_\_ / \_\_\_\_ Transfer the cryovials to a liquid nitrogen freezer, record the names and locations of all stored cell lines in a log book.
- \_\_\_\_ / \_\_\_\_ / \_\_\_\_ Thawing one cryovial after one week to make sure the cells freezing is success.

\* In general, one well of 24-well plate of hybridomas was freedded to 1 cryovials, one T25 flask of hybridomas was freezed to 1~3 cryovials, and one T75 flask of hybridomas was freezed to ~5 cryovials.

### Reference:

*John E. C., Barbara E. B., David H. M., Ethan M.S., and Warren S. (eds.) 2009. Current Protocol in immunology. John Wiley and Sons. Inc.*

Date	Operator	QC