TC4-SOP 0-5 Thawing cells

☐ Myeloma ☐ Hybridoma **Project Name:** _____

MATERIALS:

- **10% (FBS) DMEMX**
- **T25 or T80 Flasks**
- **50 mL conical tube**
- **Centrifuge**
- **37℃ water bath**
- **CO_2 incubator**
- **Laminar flow**
- **Pipetman P1000**

METHODS:

1. 10 min before the operation：
   - Warm 10% DMEMX in 37℃ water bath
   - Wipe the culture hood with 75 % ethanol, then turn on the UV light until the operation.
   - Label Flask(s) with name of cell line, thawing date, and operator.
2. Retrieve cryovial containing cells from storage area (−80℃ or liquid nitrogen freezer) and place on liquid nitrogen to keep the temperature (−196℃). Thaw cells by placing bottom half of cryovial in a 37℃ water bath (do not submerge completely). Swirl cryovial gently until cells are thawed.
3. Take cryovial to tissue culture hood and wipe vial with 75% ethanol. Gently add 1 mL 10% DMEMX and resuspend the cells using a 1 mL tip.
4. Transfer the cells to a sterile 15 mL or 50 mL tube. Add 10% DMEMX to 15 mL.
5. Cap the tube, centrifuge 5 min at 900 rpm, room temperature, and discard supernatant.
6. Gently resuspend cells and add 10% DMEMX to 15 mL, repeat step 5.
7. Resuspend cells and add 10% DMEMX to 5 mL, transfer to a T25 flask. Cap the flask, and inspect cells using an inverted microscope for morphology.
8. **Loosen the cap** of flask and move it to CO_2 incubator.
9. Next day, inspect cells for morphology.

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.

Note:

日期	操作者	QC

TechComm 100319
HJL Original
HJT Modified