TC4-SOP 1-3 Cell Fusion

Project Name: _____

MATERIALS:

- **DMEM (HyClone SH30022.02) 200 mL**
- **15% (FBS) DMEMX 50 mL with 50X HAT (Sigma H-0262) 1 mL**
- **Polyethylene Glycol 1500 (PEG 1500) (Roche 783 641) 0.7 mL**
- **Myeloma cell (SP2/0-Ag14) in one T75 Flask, ~10^7 cells**
- **50 mL tube ×4**
- **Styrofoam dissection board ×1**
- **Pin ×10**
- **Timer ×1**
- **Syringe 27 GX1/2" ×2**
- **Petri dishes ×3**
- **96 Well Plates ×3**
- **500 mL beaker**
- **Sterile surgical instrument ×1**
- **12-channel pipette**

METHODS:

☐ **Before fusion：**

- One day before fusion, change 10% DMEMX medium and reduce half of myeloma in T75 flasks. (Date ____/____/____ Time ____:____).
- 1 hr before fusion, check the materials and turn on the UV light until the operation.
- 30 min before fusion, warm 500 mL of Q water, 200 mL DMEM and 50 mL 15% DMEMX with HAT in water bath.
- 1 min before fusion, prepare 3 petri dishes filled with prewarmed DMEM.

☐ **Use CO_2 to sacrifice boosted mouse**, wet the fur with 75% ethanol, move animal to the laminar flow hood, and use pins to fix mouse on the styrofoam dissection board coverd aluminum foil.

☐ **Open the thoracic cavity**, use a syringe with needle to collect blood (~0.5ml) from the heart.

☐ **Open the abdominal cavity and gently pull and cut the spleen**. Do not rip or cut any viscus in the abdominal cavity. Transfer spleen to a sterile petri dish filled with DMEM, remove the connective tissue on the spleen. Rinse the spleen in another petri dish filled with DMEM.

☐ At the last petri dish, **use a syringe with needle to stab the spleen and pump the DMEM medium into spleen**. Transfer spleen cell suspension to a sterile 50 mL tube and add DMEM to 30 mL. Transfer the myeloma cells from T75 flasks to another 50 mL tube and add DMEM to 30 mL. Simultaneoulsy centrifuge both tubes 10 min at 900 rpm (200g).

☐ Decant supernatant and disrupt pellet. Separately add DMEM to 30 mL and count cell numbers using a hemocytometer. Simultaneoulsy centrifuge both tubes 10 min at 900 rpm (200g).

- Splenocytes (5/6): _____ X 10^4 cell； Myeloma (1/6): _____ X 10^4 cell

☐ Decant supernatant and disrupt pellet. Mix both cells at c. 1:5 ratio (myeloma : spleen cells) in a 50 mL conical tube and add DMEM to 30 mL. Centrifuge 10 min at 900 rpm (200g). While cells are in the centrifuge, move the 500 mL beaker containing 37℃ Q water to the culture hood.

☐ Decant supernatant and disrupt pellet. Place the tube in 37℃ Q water. Add **0.7 mL PEG** in **1 min**, shake the tube after each drop. **Shake hard for 1 min**. Add **2 mL DMEM** in **2 min**, stirring after each drop. Then add **8 mL DMEM** in **2 min**. Centrifuge 8 min at 800 rpm. Decant supernatant and disrupt pellet. Add DMEM to 30 mL.

☐ Centrifuge 8 min at 800 rpm. Discard supernatant, gently resuspend the pellet. Add 45 mL DMEMX-HAT。

☐ Add 150 µl of suspension to each well of three 96-well plates. Place all plates in humidified 37℃, 7% CO_2 incubator.

Date	Operator	QC