boost times: _____ dose: _____ μg/mouse

TC4-SOP 1-1m Immunize mice

Project Name: _____

MATERIALS:

- Mouse x ____
  - ☐ BALB/c Birth Date: ____/____/____
- 3-way stopcock (Nipro)
- Antigen: _____
  - ☐ _____ μg /μL, _____ μL ☐ _____ μg
  - ☐ Powder ☐ Gel ☐ liquid, solvent _____
- Adjuvant
  - ☐ Complete Freund's Adjuvant (CFA) ☐ Incomplete Freund's Adjuvant (IFA)
  - ☐ other: _____ ☐ None

- Sterile syringes (Terumo, 27 GX1/2", 1 mL)
- Paraffin membrane (Parafilm)
- PBS buffer (1X)

METHODS:

☐ For first immunization, prepare antigen using 100 μg protein or peptide per animal to be immunized. For subsequent boosters, using 50 μg antigen. Add 1X PBS to 250 μL. (Antigen _____ μL, add 1X PBS to 250 μL)

☐ Add antigen into a sterile syringe using tips. Carefully clean the end of syringe without needle, connect syringe to a 3-way stopcock. Seal the connection part by using parafilm.

☐ Add a volume of CFA[1]/IFA[2] equal to the antigen volume into another syringe using tips. Carefully clean the end of syringe, connect syringe to the 3-way stopcock. Seal the connection part by using parafilm.
  1. Completely resuspend CFA to disperse the *Mycobacterium tuberculosis* bacilli.
  2. CFA is for first booster, IFA is for subsequent booster.

☐ Turn the 3-way stopcock, **discharging antigen into adjuvant*.**
  * Add one drop of adjuvant into the syringe plunger.

☐ Discharging back and forth half an hour until a thickened mixture results*.
  * A stable emulsion will not disperse when a drop of it is placed in water, or looks like the tooth paster.

☐ Transfer all of the adjuvant/antigen emulsion to one syringe. Attach a sterile 27G needle to the syring containing the emulsion.

☐ Inject emulsion intraperitoneally into the animal using <500 μL/mouse.

☐ **Boost animal after 14 days and bleed to monitor the antibody titer.**
  * If cell fusion is planned for 3~7 days after boosting, immunize with antigen alone in 1X PBS. If a fusion is not immediately planned, boost the animal with antigen emulsified in IFA.

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