



Title: *Hybridoma Generation: Inactivated Gradient-
Enriched Coronavirus (CV)*

No: RTLP-GL-Ab-17

Location:
Old CCRC Tripp Lab

Approval Date:
10 September 2004

Supersedes Date:

Materials:

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|-----------|--|-----------------------------|------------------|
| •Lab coat | •Phosphate Buffered | •1 mL Leur- lock | •Pipettes |
| •Gloves | Saline (PBS) | syringes | •Pipetteman |
| | •Inactivated gradient-
enriched coronavirus
(CV) | •25 gauge needle | •Pipette Aid |
| | | •Adjuvant:
TiterMax Gold | •Pipetteman tips |

Procedure:

1. Resuspend 1-50 mg of CV protein in PBS.
2. Using a 1 mL Leur-lok syringe, draw up CV protein solution.
3. Prepare adjuvant (TiterMax Gold) per manufacturers instructions, then draw up an equal volume of adjuvant in a **separate** syringe
4. Attach 3-way stopcock, and emulsify the CV protein and adjuvant.
5. Transfer CV-emulsification to a single 1 mL syringe.
6. Eye/tail bleed naïve mice for analysis.
7. Inject CV-emulsification intra-peritoneally into naïve mouse using 0.2 ml emulsification/mouse
8. Let the mouse rest 14 days.
9. At day 14 post-treatment, eye/tail bleed mouse for analysis
10. Repeat steps 1-5 and boost animal with the same dose as in step 7.
11. Let mouse rest 7 days.

12. At day 21 post-boost, eye/tail bleed
13. Boost animal with the same dose as in step 7.
14. At day 24, harvest spleen for fusion.

Author	Management Approval/Date	Effective Date