



Title: *Hybridoma Generation: Sera Screening*
For antibodies against Inactivated Gradient-Enriched
Coronavirus (CV) or Adenovirus (Ad) expression vector-
spike (Ad-S) or Ad-nucleoprotein (Ad-N)

No: RTLP-GL-Ab-16

Location:
Old CCRC Tripp Lab

Approval Date:
10 September 2004

Supersedes Date:

Materials:

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- Lab coat
- Gloves
- RTLP-GL-22 for ELISA
- Test sera from mouse
- Control Antigens:
Inactivated gradient-enriched coronavirus (CV) **or**
Adenovirus (Ad) expression vector-spike (Ad-S) **or**
Adenovirus (Ad) expression vector-nucleoprotein (Ad-N)
- Horse radish peroxidase (HRPO) α -mouse 2^o antibody
- Positive Control 229E
- HRPO- α -rabbit antibody
- TMB substrate

Procedure:

I. Sera Screening Assay:

Screening is performed by ELISA: see RTLP-GL-30 for protocol

The following substitution may be made:

1. Coat wells of Immulon plate with CV or control antigen.
2. Wash 3x and block.
3. Wash 3x, and add dilution series of collected sera and positive control, i.e 229E
4. Incubate, wash 3x, add HRPO-anti-mouse secondary antibody to sera wells, or HRPO-anti-rabbit antibody to 229E well.
5. Incubate, wash 3x, add TMB substrate, read absorbance.

NOTE: Do not perform fusion until sera screening is complete – must see rise in titer.

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Author	Management Approval/Date	Effective Date