

Supplies and Reagents:

- **Tetracaine Hydrochloride Ophthalmic solution (Pick up from LAH)**
- **Pierce Rapid ELISA Mouse mAB Isotyping Kit- Invitrogen, #37503**

I. Collecting Serum – Retro Orbital Eye Bleeds

1. Place one drop of Tetracaine Hydrochloride Ophthalmic Solution USP on the eye and blot with paper towel.
2. Wait five minutes to allow the anesthetic to set in.
3. Bleed the mouse to extract two capillary tubes of blood and place in labeled test tubes.
4. Centrifuge the samples at 16.1 rcf for 3 minutes.
5. Using a pipette, extract the serum from the test tubes and transfer to new test tubes.
6. Freeze the serum at -20°C.

II. ELISA Assay-

1. Remove the serum samples from the -20°C freezer and let them thaw on ice.
2. Begin preparation while the samples thaw by dissolving the BupH Tris Buffered Saline Powder into 500mL of ultrapure water.
3. Make the wash buffer by adding 10mL of the wash buffer to 290mL of ultrapure water.
4. To begin the dilutions for serum samples, add 1µL of the serum sample to 10mL of Tris Buffered Saline (TBS), creating a dilution of 1:10,000.
 - a. For a dilution of 1:50,000, take 1mL of the 1:10,000 dilution of each sample and add 4mL of TBS.
 - b. For a sample dilution of 1:100,000, take 1mL of the 1:50,000 dilution and add 1mL of TBS.
5. To begin antibody isotyping, place 50 µL of the correctly diluted serum sample in each pre-coated well of the plate.
6. Add 50 µL of Goat anti-mouse IgG + IgA + IgM HRP Conjugate to each well of the 8-well strip and gently tap to mix.
7. After the plate is assembled, it must incubate at room temperature for one hour.
8. Empty the contents of the plate.
9. Use a pipette to wash the plate four times with 50 µL of wash buffer.
10. Add 75 µL of stop solution. The positive response wells should change from blue to yellow.
11. Measure the plate with a spectrophotometer at 450 nm. An absorbance reading of greater than or equal to 0.2 is a positive response.