Standard ELISA

1. Coat 96 well flat-bottom plates (Nunc- Fisher Scientific Pittsburgh, PA) with purified capture antibody overnight at 4ºC.
2. Coat antibody using sterile PBS at 50uL/well.
    a. IL-2, IL-4, IL-5, IL-10, and IFN-γ at final concentration of 2ug/mL (Pharmingen).
    b. TNFα at final concentration of 4ug/mL.
3. Block plate with 1% BSA/PBS-Tween (Sigma) for 30 minutes.
4. Wash plate 3 times with PBS/Tween; then, add serum/supernatant samples and incubate overnight at 4ºC.
5. Add standards at a concentration of 50ng/mL and titrate to a two-fold dilution.
6. Wash plate 3 times in PBS/Tween and add biotinylated detection antibody (Pharmingen) at 2ug/mL for all cytokines.
7. Incubate detection antibody overnight at 4ºC.
8. Wash plate 3 times with PBS/Tween and add 1:1000 dilution of horseradish peroxidase (Vector Laboratories, Burlingame, CA) in 1% PBS/Tween at 50uL/well.
9. Incubate at room temperature for 1 hour in the dark.
10. Wash plate 3 times with PBS/Tween and add ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt, Sigma, St. Louis, MO) at 50uL/well.
11. Read plate at 405nm on microplate reader (BioRad Model 3550).