

- 4) Discard the primary antibody solution and wash 3X with TST.
- 5) Add 50 μ l of diluted secondary antibody conjugated to Alkaline Phosphatase. I use a 1:500 dilution of rabbit anti-mouse IgG (Zymed). Incubate at room temperature for 1 hour. About 15 min before the end of the incubation start dissolving the p-nitrophenyl phosphate substrate tablets. For each ELISA plate dissolve 1 tablet in 5 ml of AP Buffer.
- 6) Discard the secondary antibody solution and wash 3X with TST.
- 7) Add 50 μ l per well of the p-nitrophenyl phosphate solution and incubate at room temperature for 10-30 min. Stop the reactions by adding 50 μ l of 0.1 M EDTA pH8.0. For screening monoclonals it is best to let them go until all of the positives are clearly visible, however if you want to be quantitative it is important not to let them get intensely yellow or they will be off scale--the plate reader is most accurate when the wells have just begun to clearly turn yellow.