Antibody binding experiment

1. Prepare 8ml fluorescein stock solution of concentration 1.2nM.
2. Prepare 1ml antibody 4-4-20 solution of concentration 12nM.
3. Take 0.5ml solution 2 into a new test tube. Add 0.5 ml buffer into the new test tube. Mix thoroughly.
4. Repeat step 3 for 10 times. Then we have 12 antibody solutions with concentration of a factor 2 less successively.
5. Put 0.5 ml fluorescein stock solution 1 into each of the 12 test tubes and mix.
6. Select an excitation wavelength of 470nm and an emission wavelength range of 500-600nm.
7. Measure the buffer and 0.6nM fluorescein emission.
8. Start with lowest concentration of antibody samples. Measure the emission spectrum.
9. For each emission spectrum, correct the background by subtracting the background spectrum. Then integrate the spectrum to get the total fluorescent intensity. Record the value of total intensity.
10. Analyze data:
    a. Calculate the fluorescence quenching percentage of each fluorescein-antibody sample with respect to pure fluorescein $Q = \left(1 - I \middle/ I_{\text{Pure fluorescein}}\right) \times 100\%$.
    b. Calculate the fluorescence ratio r between the completely bound fluorescein and free fluorescein $r = I_{\text{Completely Bound}} \middle/ I_{\text{Pure fluorescein}}$.
    c. Use the ratio r to calculate the free ligand concentration with the formular $[L] = [L]_T \dfrac{\left(I - I_{\text{completely bound}}\right) \Big/ I_{\text{Pure fluorescein}}}{1 - r}$.
    d. Calculate the free antibody binding sites concentration by subtracting the total enzyme concentration by the bound antibody concentration $[\text{ABS}]_{\text{Free}} = [\text{ABS}]_{\text{Total}} - \left([L]_T - [L]\right) \times 2$.
    e. Plot quenching Q as a function of $[\text{ABS}]_{\text{Free}}$ and fit to the function $Q = \dfrac{a \times [\text{ABS}]_{\text{Free}}}{K_d + [\text{ABS}]_{\text{Free}}} + b$ to obtain K_d.

Polarization Experiment

1. Measure the anisotropy of free fluorescein, partly bound fluorescein, and completely bound fluorescein.