



My antibody concentration is less than 2 mg/ml. Must I concentrate it before performing a conjugation?

Not necessarily. The efficiency of conjugation and the degree of aggregation of the conjugate sometimes, but not always, depend on the concentration at which the conjugation is performed. In many instances an antibody will conjugate acceptably well at a lower concentration.

Another limiting factor on concentration is the volume of reduced antibody that is applied to Desalting Column A. Since the maximum sample volume for this column is 2 ml, a 1-mg conjugation reaction requires a minimum antibody concentration of 0.5 mg/ml. Despite this limitation, a still lower antibody concentration may suffice if the reaction is scaled down (*i.e.* a one-half mg reaction can be tried with an antibody concentration of down to about 0.25 mg/ml).

If the resulting conjugate doesn't give as bright a signal in your application as desired, then try concentrating the antibody and repeat the conjugation.