

Hypothetical Plate Layout (48 well plate)

3.000 Std.	3.000 Std.	Control 1	Control 2	Control 3	Control 4
1.000 Std.	1.000 Std.	Control 1	Control 2	Control 3	Control 4
0.333 Std.	0.333 Std.	Control 1	Control 2	Control 3	Control 4
0.111 Std.	0.111 Std.	Exper. 1	Exper. 2	Exper. 3	Exper. 4
0.037 Std.	0.037 Std.	Exper. 1	Exper. 2	Exper. 3	Exper. 4
0.012 Std.	0.012 Std.	Exper. 1	Exper. 2	Exper. 3	Exper. 4
Zero	Zero				

Hypothetical Results (48 well plate)

		c					
A		0.172	0.177	1.543	1.321	1.064	1.153
		0.491	0.354	1.554	1.203	1.031	1.206
		0.740	0.660	1.549	1.262	0.999	1.195
		1.233	1.203	0.336	0.362	0.455	0.659
		1.616	1.511	0.348	0.401	0.478	0.598
		1.799	1.675	0.395	0.389	0.423	0.623
B		1.866	1.818	----	----	----	----
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How to do calculations:

- 1) Compute the average optical density (OD) for all duplicate / triplicate wells (standards / unknowns)

Examples:

3.000 standard: $(0.172 + 0.177) / 2 = \mathbf{0.175}$ (duplicates – Box A)

Zero well: $(1.866 + 1.818) / 2 = \mathbf{1.842}$ (duplicates – Box B)

Control 2: $(1.321 + 1.203 + 1.262) / 3 = \mathbf{1.262}$ (triplicates – Box C)

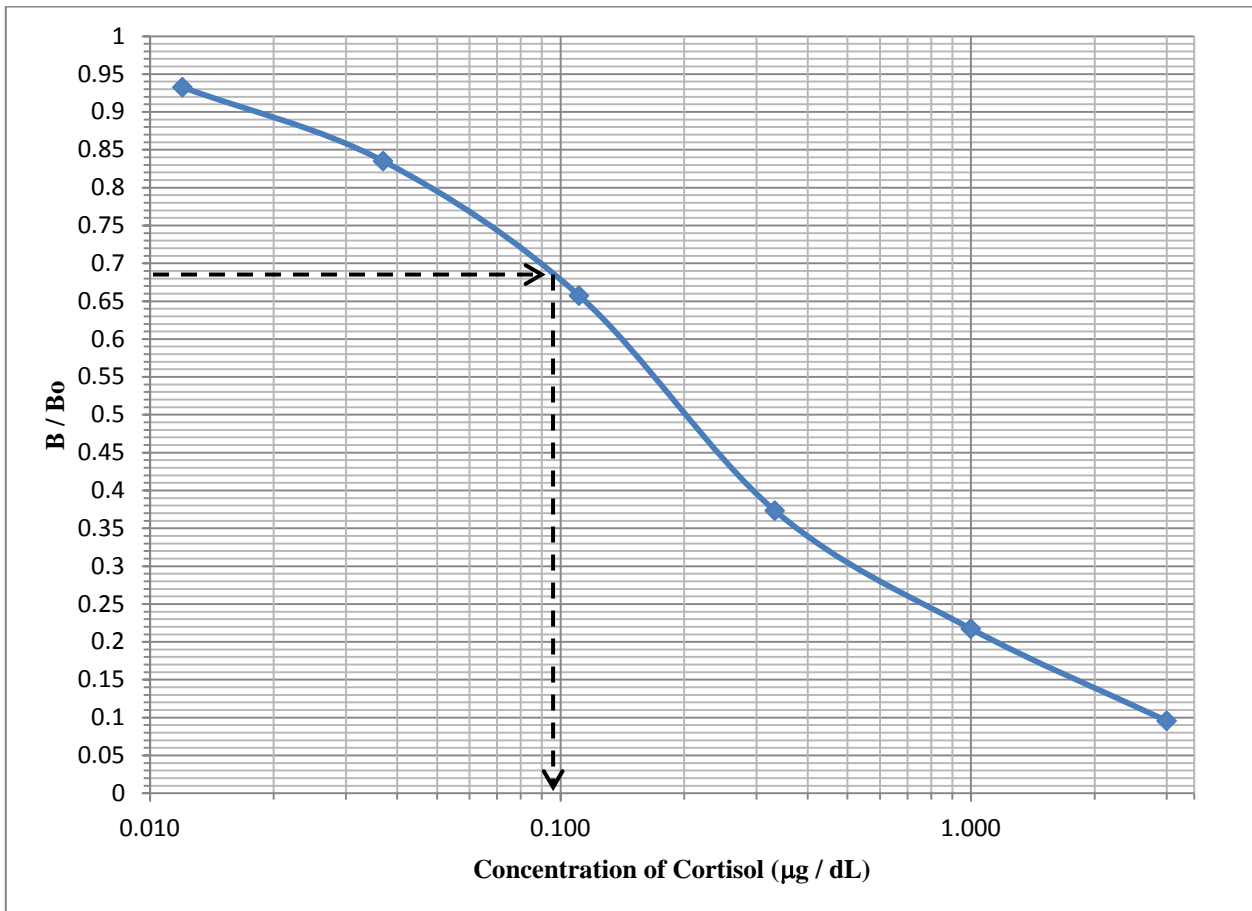
- 2) Calculate the percent bound of the conjugate (B / Bo) for each standard and unknown by dividing the average OD of each standard / unknown (B) by the average OD for the zero wells (Bo).

Examples:

Percent bound for 3.000 standard: $0.175 / 1.842 = \mathbf{0.093}$

Percent bound for Control 1: $1.262 / 1.842 = \mathbf{0.685}$

- 3) Determine the concentrations of the unknowns through the use of a standard curve calculated with the six standards. The standard curve should be drawn using the Excel worksheet available on the class webpage.



- 4) To determine the unknown concentrations, find the point on the curve for the appropriate percent bound (B / B_0) for each unknown on the Y-axis and look to see what concentration it represents on the X-axis.

Example:

Cortisol concentration of Control 2: ~ **0.10 $\mu\text{g} / \text{dL}$**

- * Do not worry that you will not be precise in determining the concentration; it is more important to understand how the process works than to have the exact number. If we were interested in precision, then we would insert the B / B_0 into the mathematical equation for the standard curve line – this is the way it is done in research where precision is required.