ADIFAB Information Sheet

▪ MEASUREMENT INFORMATION

- To measure the aqueous free (unbound) fatty acid concentration of a sample with ADIFAB, the ratio (R) of fluorescence emission at 505 and 432 nm (upon excitation at 386 nm) is measured and used in the equations below.

- **Note: Ro and Rmax are instrument dependent**. **YOU NEED TO DETERMINE THE RMAX BY THE FOLLOWING METHOD:** The "calibration" values were obtained from multiple binding isotherms measured at 37°C with a specific fluorometer. **The Ro calibration value is 0.20 and the Rmax Calibration Value is 9.9**. This Rmax calibration value is different than the published value of 11.5 due to changes in the probe composition that improve performance.

- **Rmax for the measurement is calculated using the following equation:**

$Rmax = (Ro_{measured} / Ro_{calibration}) \bullet R_{max}$ Calibration Value

No correction is needed for Kd or Q (19.5).

- **FFAu** (in Nanomolar (nM)) can then be calculated using the following equation:

$$[\mathrm{FFAu}] = \mathrm{K_d} \bullet 19.5 \bullet \frac{(\mathrm{R} - \mathrm{R_o})}{(R\max - \mathrm{R})}$$

Fatty acid bound (in micromolar) to ADIFAB can be calculated as:

$$[\mathrm{ADIFAB_{bound}}] = \frac{[\mathrm{ADIFAB_{total}}] \bullet 19.5 \bullet (\mathrm{R} - \mathrm{R_o})}{R\max - \mathrm{R} + 19.5 \bullet (\mathrm{R} - \mathrm{R_o})}$$

where R is the ratio of the fatty acid sample with ADIFAB and Ro is the ratio of ADIFAB in the absence of fatty acid

$$\mathrm{R} = \frac{\mathrm{I_{505}} - \mathrm{I_{505}^{blank}}}{\mathrm{I_{432}} - \mathrm{I_{432}^{blank}}} \qquad \mathrm{Ro} = \frac{\mathrm{I_{505}^{o}} - \mathrm{I_{505}^{blank}}}{\mathrm{I_{432}^{o}} - \mathrm{I_{432}^{blank}}}$$

K_ds (in μM) for fatty acid binding to ADIFAB at various temperatures

Temperature	Laurate	Myristate	Palmitate	Oleate	Linoleate	Linolenate	Arachidonate
(°C)							
5	12.3	2.54	0.20	0.17	0.48	1.31	0.72
10	12.5	2.67	0.22	0.19	0.54	1.47	0.82
15	12.8	2.80	0.24	0.20	0.60	1.63	0.94
20	13.1	2.93	0.26	0.22	0.67	1.81	1.07
25	13.4	3.07	0.28	0.23	0.74	2.00	1.22
30	13.6	3.21	0.31	0.25	0.82	2.21	1.38
35	13.8	3.30	0.33	0.27	0.90	2.42	1.55
37	14.0	3.40	0.34	0.28	0.94	2.51	1.62
40	14.2	3.48	0.36	0.29	0.99	2.65	1.73
45	14.4	3.62	0.39	0.31	1.09	2.90	1.94
50	14.7	3.77	0.41	0.33	1.18	3.15	2.16

Contact us about a service for determining the unbound FFA Profiles of a given sample (up to 12 different fatty acids can be quantified)

For additional information please contact FFA Sciences at (858)-455-3776 or info@ffasciences.com.

ADIFAB is intended for research use only.