

Determining the ADIFAB Ratio

Synopsis

In the absence of unbound free fatty acids, the ADIFAB probe fluoresces in the blue at 432 nm. In the presence of unbound free fatty acids, the emission shifts to the green with a peak at 505 nm. The ratio of fluorescence at 505 nm and 432 nm allows the concentration of unbound free fatty acids to be determined. This protocol outlines how to calculate and use the ADIFAB ratio (R).

Procedure

R_0 is the ADIFAB ratio *without* fatty acid present. To determine R_0 , excite a cuvette containing measuring buffer only (20 mM HEPES, 140 nM NaCl, 5 mM KCl, 1 mM Na_2HPO_4 , at pH 7.4) at 386 nm and measure the fluorescence intensities at 505 and 432 nm. These are the blank intensities. Then add 0.2 μM ADIFAB to the cuvette, mix gently *avoiding bubble formation*, and measure the emission at 505 and 432 nm. Substitute the intensities into the R_0 expression:

$$R_0 = \frac{I_{505}^o - I_{505}^{\text{blank}}}{I_{432}^o - I_{432}^{\text{blank}}}$$

To measure R, add an aliquot of a solution containing fatty acid to the cuvette and mix. Measure the intensities at 505 and 432 nm and substitute them into the R expression below. If the fatty acid solution contributes significant scattering or fluorescence, remeasure the blank intensities *with* fatty acid.

$$R = \frac{I_{505} - I_{505}^{\text{blank}}}{I_{432} - I_{432}^{\text{blank}}}$$

Substitute R_0 and R into Eq. (1), along with the appropriate dissociation constant, K_d , from Table 1 to determine the free fatty acid (FFA) concentration.

$$[\text{FFA}] = K_d \cdot 195 \cdot \frac{(R - R_0)}{(115 - R)} \quad (1)$$

To determine the fatty acid concentration bound to ADIFAB, use Eq. (2).

$$[\text{ADIFAB}_{\text{bound}}] = \frac{[\text{ADIFAB}_{\text{total}}] \cdot 195 \cdot (R - R_o)}{115 - R + 195 \cdot (R - R_o)} \quad (2)$$

Table 1. K_d values for fatty acid binding to ADIFAB (in μM).

Temperature (°C)	Laurate	Myristate	Palmitate	Oleate	Linoleate	Linolenate	Arachidonate
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

All K_d values measured in HEPES measuring buffer: 20 mM HEPES, 140 nM NaCl, 5 mM KCl, 1 mM Na_2HPO_4 , at pH 7.4. To determine constants in an alternative buffer or at a different temperature see [Determining ADIFAB Fatty Acid Dissociation Constants: \$K_d\$](#) .