Determining the ADIFAB2 Ratio

Synopsis
In the absence of unbound free fatty acids, the ADIFAB2 probe fluoresces in the blue at 457 nm. In the presence of unbound free fatty acids, the emission shifts to the green with a peak at 550 nm. The ratio of fluorescence at 550 nm and 457 nm allows the concentration of unbound free fatty acids to be determined. This protocol outlines how to calculate and use the ADIFAB2 ratio (R).

Procedure
R_0 is the ADIFAB2 ratio *without* fatty acid present. To determine R_0, excite a cuvette containing measuring buffer (20 mM HEPES, 140 nM NaCl, 5 mM KCl, 1 mM Na_2HPO_4, at pH 7.4) at 375 nm and measure the fluorescence intensities at 550 and 457 nm. These are the blank intensities. Then add 0.5 μM ADIFAB2 to the cuvette, mix gently *avoiding bubble formation*, and measure the emission at 550 and 457 nm. Substitute the intensities into the R_0 expression:

$$R_0 = \frac{I_{550}^0 - I_{550}^{blank}}{I_{457}^0 - I_{457}^{blank}}$$

To measure R, add an aliquot of the fatty acid solution to the cuvette and mix. Measure the intensities at 550 and 457 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.

$$R = \frac{I_{550} - I_{550}^{blank}}{I_{457} - I_{457}^{blank}}$$

Substitute R_0 and R into Eq. (1), along with the appropriate K_d, Q and R_{max} from Table 1 to determine the free fatty acid (FFA) concentration.

$$[FFA] = K_d \bullet Q \bullet \frac{(R - R_o)}{(R_{max} - R)} \quad (1)$$

To determine the fatty acid concentration bound to ADIFAB2, use Eq. (2).

$$[ADIFAB2_{bound}] = \frac{[ADIFAB2_{total}] \bullet Q \bullet (R - R_o)}{R_{max} - R + Q \bullet (R - R_o)} \quad (2)$$

In these expressions K_d refers to the ADIFAB2 dissociation constant, Q refers to the intensity of ADIFAB2 at 457 nm in the *unbound* state (no fatty acid present) divided by the intensity at 457 nm in the *bound* state (completely saturated with fatty acid), and R_{max} refers to the ADIFAB2 ratio (550/457) in the completely bound state.

Table 1. Fluorometric constants for fatty acid binding to ADIFAB2 at 22°C.

Fatty Acid	Q	K_d (nM)	R_{max}
Palmitate (16:0)	6.6	21.4	1.16
Stearate (18:0)	6.6	9.3	1.19
Oleate (18:1) 9 cis	5	32	0.762
Linoleate (18:2) 9,12 cis	4	101	0.727
Linolenate (18:3) 9,12,15 cis	5	237	0.798
Arachidonate (20:4)	5	167	0.912

All 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 ADIFAB2 Fatty Acid Binding Constants.