Determining the Unbound Free Fatty Acid Concentration in Serum Samples

Synopsis
ADIFAB can be used to determine the unbound free fatty acid (FFA) concentration in serum by measuring the value of the ADIFAB ratio with and without serum present.

Procedure
For details on measuring the ADIFAB ratio and calculating [FFA] see Determining the ADIFAB Ratio. To determine R_0, add 0.2 μM ADIFAB and 6 μM fatty acid free bovine serum albumin (BSA) to a cuvette containing buffer, and measure the fluorescence ratio (505/432 upon excitation at 386 nm). To measure the R value of a serum sample, add 0.2 μM ADIFAB and 1% serum (by volume) to a separate cuvette containing buffer and measure 505/432. The 100 fold dilution of the serum yields an albumin concentration of ~6 μM, the same as used to determine R_0. This dilution does not affect [FFA] since [FFA] is buffered by the $[FA]_{total}$:albumin ratio. To calculate [FFA] substitute R and R_0 into Eq. (1):

$$[FFA] = K_d \bullet 19.5 \bullet \frac{(R - R_o)}{(11.5 - R)} \quad (1)$$

For serum, K_d is a weighted average of the K_ds for the various fatty acids present in serum and is equal to 0.44 μM at 37°C.

Notes
- Because the difference between R and R_0 is very small (less than 0.005 for serum from a healthy donor), to insure accuracy, average at least 5 measurements of R and R_0 (which can be done automatically on most fluorometers).
- When measuring multiple serum samples, we advise taking 2 R_0 measurements, 8-10 serum sample measurements, 2 R_0, 8-10 serum samples, etc.
- It is important that the serum samples contain little or no hemoglobin because hemoglobin preferentially absorbs the 432 nm fluorescence intensity, giving erroneously high FFA values.
- We recommend using ADIFAB2 for serum measurements because it is not affected by hemoglobin and it has greater sensitivity in this range.

Example
6 μM BSA was added to a cuvette containing 1.5 ml buffer (20 mM HEPES, 140 nM NaCl, 5 mM KCl, 1 mM Na_2HPO_4, at pH 7.4 and 37°C) and blank intensities at 432 and 505 nm (upon excitation at 386 nm) were measured. 0.2 μM ADIFAB was added to the cuvette, and after gently mixing the solution, the R_0 value was measured and found to be 0.2500. To another cuvette, 15 μl of a serum sample were added and blank

intensities were measured. The R value was measured and found to be 0.2530 after 0.2 μM ADIFAB was added. Using Eq. (1) and K_d = 440 nM, the FFA concentration was found to be 2.3 nM.