

Determining the Unbound Free Fatty Acid Concentration in Serum Samples

Synopsis

ADIFAB2 can be used to determine the unbound free fatty acid (FFA) concentration in serum by measuring the value of the ADIFAB2 ratio with and without serum present.

Procedure

For details on measuring the ADIFAB2 ratio and calculating [FFA] see [Determining the ADIFAB2 Ratio](#). To determine R_0 , add 0.5 μM ADIFAB2 and 6 μM fatty acid free bovine serum albumin (BSA) to a cuvette containing buffer, and measure the fluorescence ratio (550/457 upon excitation at 375 nm). To measure the R value of a serum sample, add 0.5 μM ADIFAB2 and 1% serum (by volume) to a separate cuvette containing buffer and measure 550/457. The 100 fold dilution of the serum yields an albumin concentration of $\sim 6 \mu\text{M}$, the same as used to determine R_0 . This dilution does not effect [FFA] since [FFA] is buffered by the $[\text{FA}]_{\text{total}}$:albumin ratio. To calculate [FFA] substitute R and R_0 into Eq. (1):

$$[\text{FFA}] = K_d \cdot Q \cdot \frac{(R - R_0)}{(R_{\text{max}} - R)} \quad (1)$$

For serum at 22°C, $K_d = 45.5 \text{ nM}$, $Q = 5$ and $R_{\text{max}} = 0.925$.

Notes

- Because the difference between R and R_0 is very small (less than 0.01 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.

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 22°C) and blank intensities at 457 and 550 nm (upon excitation at 375 nm) were measured. 0.5 μM ADIFAB2 was added to the cuvette, and after gently mixing the solution, the R_0 value was measured and found to be 0.0870. 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 0.0950 after 0.5 μM ADIFAB2 was added. Using Eq. (1) and the constants above, the FFA concentration was calculated to be 2.2 nM.