

## 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  $\mu\text{M}$  ADIFAB2 and 6  $\mu\text{M}$  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  $\mu\text{M}$  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  $\mu\text{M}$  BSA was added to a cuvette containing 1.5 ml buffer (20 mM HEPES, 140 nM NaCl, 5 mM KCl, 1 mM  $\text{Na}_2\text{HPO}_4$ , at pH 7.4 and 22°C) and blank intensities at 457 and 550 nm (upon excitation at 375 nm) were measured. 0.5  $\mu\text{M}$  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  $\mu\text{l}$  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  $\mu\text{M}$  ADIFAB2 was added. Using Eq. (1) and the constants above, the FFA concentration was calculated to be 2.2 nM.