



## ADIFAB2 Information Sheet

For additional information please see Protocols at [www.ffasciences.com](http://www.ffasciences.com).

- To measure the aqueous free (unbound) fatty acid concentration of a sample with ADIFAB2, the ratio (R) of fluorescence emission at 550 and 457 nm (upon excitation at 375 nm) is measured.
- **Note: Ro and Rmax are instrument dependent.** The “calibration” values were obtained from multiple binding isotherms with a specific fluorometer. **The Ro calibration value is 0.082 for 22C. The Rmax Calibration Value is fatty acid dependent and several are listed below.**
- To use the equations below for determining the free and bound FFA concentrations you must measure Ro on your instrument (Measure the ADIFAB Ro in HEPES + BSA with the same BSA concentration as the sample (e.g. the standard))
- **Rmax for the measurement is calculated using the following equation:**

$$R_{\max} = (R_{\text{measured}} / R_{\text{calibration}}) \bullet R_{\max} \text{ Calibration Value}$$

- FFAu can then be calculated using the following equation:

$$[\text{FFAu}] = K_d \bullet Q \bullet \frac{(R - R_o)}{(R_{\max} - R)}$$

Fatty acid bound to ADIFAB2 can be calculated as:

$$[\text{ADIFAB2}_{\text{bound}}] = \frac{[\text{ADIFAB2}_{\text{total}}] \bullet Q \bullet (R - R_o)}{R_{\max} - R + Q \bullet (R - R_o)}$$

where R is the ratio of the fatty acid sample with ADIFAB2:

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

and Ro is the ratio of ADIFAB2 in the absence of fatty acid:

$$R_o = \frac{I_{550}^o - I_{550}^{\text{blank}}}{I_{457}^o - I_{457}^{\text{blank}}}$$



## ADIFAB2 Information Sheet continued

At 22°C the constants  $K_d$ , Q and  $R_{max}$  have been determined for several fatty acids:

FA	Kd (nM)	KdQ	$R_{max_{calibration}}$ Value
AA	135.8	782.4	1.048
LA	93.3	383.2	0.673
LNA	239	1985.4	1.252
OA	22.8	120.6	0.783
PA	18.3	201.5	1.54
SA	7.6	38.0	0.838
Plasma/Serum	45.5	227.5	0.925
POA	257	1193.4	0.865
MA	390	1600.4	0.756
EPA	340	1869.8	1.068
DHA	253	2108.1	1.809
VA	48.9	358.1	1.146

Contact us about a service for determining the unbound FFA Profiles of a given sample (up to 12 different fatty acids can be quantified)

ADIFAB2 is intended for research use only. For additional information please contact FFA Sciences at (858)-455-3776 or [info@ffasciences.com](mailto:info@ffasciences.com).