Instructions for Preparation and Use of ADIFAB2

UPON ARRIVAL IMMEDIATELY:

- FREEZE (at -70°C if possible) LYOPHOLIZED ADIFAB2
  - When you reconstitute in solution do not freeze ADIFAB (keep refrigerated)
- FREEZE (at -20°C) OA:BSA STANDARD(S)- DO NOT USE A FROST FREE FREEZER
- REFRIGERATE (at 4°C) STORAGE AND MEASURING BUFFERS

1. Preparation of ADIFAB2 stock solution: Add Storage Buffer to the vial of lyophilized ADIFAB2 powder to give a final concentration of ~100 μM (approximate molecular weight of ADIFAB2 is 15000 g/mole, therefore, for 200 μg ADIFAB2 add 133 μL Storage Buffer and for 1 mg ADIFAB2 add 667 μL). **Once Storage Buffer is added to ADIFAB2, store at 4°C (don't freeze).** Storage Buffer consists of measuring buffer, 1 mM EDTA and 0.05% sodium azide. The pH at room temperature is 7.4 ± 0.1.

2. Measuring Buffer consists of 50 mM HEPES, 140 mM NaCl, 5 mM KCl, 1 mM Na_2HPO_4. pH at room temperature equals 7.4 ± 0.1. Store the Measuring Buffer at 4ºC. Recommended concentration of ADIFAB2 to be used in the measuring buffer is approximately 0.5 μM. The concentration can be increased or decreased depending on the efficiency of the fluorometer used.

3. To perform a measurement: **(a)** measure at least two or three Ro's (average the values for use in the equations listed on the ADIFAB Information Sheet). **(b)** Measure the included OA-BSA complex (discussed in 5 below) to insure your instrument is properly setup. To measure the complex add 1.5mL Measuring Buffer + 15uL OA-BSA (for a typically sized cuvette). **(c)** Mix and take a blank (no ADIFAB2) reading at the two specified wavelengths as indicated in the Information Sheet. **(d)** Add ADIFAB2 in recommended concentration (0.5μM) to cuvette and mix. Take sample reading. Apply equations included in Information Sheet to determine free fatty acid concentration. **(e)** If the result of the OA:BSA complex is correct you can move on to measuring your samples. To do so add sample to be measured to Measuring Buffer in a cuvette. Same quantities as the complex- 1.5mL Measuring Buffer + 15uL of your sample (use same equations).

4. Cuvettes made of glass or quartz are recommended for the measurement of ADIFAB2. These cuvettes must be very clean and all traces of soap rinsed away. A final rinse of ethanol and then drying under a nitrogen stream is recommended. **Plastic cuvettes made of polystyrene from Sarstedt (cat# 67.754) have been found to work well. However acrylic cuvettes have been found to leach a substance that reacts with the probe.** Cuvettes made of other materials can be easily tested by determining if the ADIFAB2 ratio changes over time. If using plates, our experience has been that black Nalgene plates (96 well catalog # 237108 & 384 well catalog # 262260) perform the best. Observe Protocol for Measuring FFA with AD2 on 96-Well Plate.

5. **IMPORTANT-** Use the included standard to check your instrument setup and measuring procedure by making a measurement using the standard (the standard is an OA-BSA complex designed to yield 5 or 50 nM (concentration noted on the vial label) unbound OA (oleate) whose BSA concentration is 600 μM). If the resulting concentration you achieve is similar to the concentration noted on the standard vial then you are making the measurements correctly and can proceed. If not, check to make sure your instrument is setup properly.

ADIFAB2 is intended for research use only. For additional information please contact FFA Sciences at (858)-455-3776.