

## Preparation and Use of ADIFAB

**When you receive your order of ADIFAB, complete the following three steps IMMEDIATELY :**

- 1. FREEZE (at -70°C if possible) LYOPHOLIZED ADIFAB**
- 2. FREEZE (at -20°C) STANDARD(S)**
- 3. REFRIGERATE (at 4°C) STORAGE AND MEASURING BUFFERS**

### **Preparation of ADIFAB stock solution:**

Add storage buffer to the vial of lyophilized ADIFAB powder to give a final concentration of  $\sim 100 \mu\text{M}$ . The approximate molecular weight of ADIFAB is 15000 g/mole; therefore, for every 200  $\mu\text{g}$  ADIFAB, add 133  $\mu\text{L}$  storage buffer and for 1 mg ADIFAB, add 667  $\mu\text{L}$ . Once storage buffer is added to ADIFAB, store at 4°C. The storage buffer consists of 50 mM TRIS, 1 mM EDTA, and 0.05% sodium azide. The pH at room temperature is  $8.0 \pm 0.1$ .

### **Measuring buffer:**

The measuring buffer consists of 20 mM HEPES, 140 mM NaCl, 5 mM KCl, and 1 mM  $\text{Na}_2\text{HPO}_4$ . The pH at room temperature equals  $7.4 \pm 0.1$ . Store the measuring buffer at 4°C. The recommended concentration of ADIFAB to be used in the measuring buffer is approximately 0.2  $\mu\text{M}$ . The concentration can be increased or decreased depending on the efficiency of the fluorometer used.

### **Cuvettes:**

Cuvettes made of glass or quartz are recommended for the measurement of ADIFAB. 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. Disposable cuvettes can also be used. Plastic cuvettes made of polystyrene from Sarstedt (cat# 67.754) have been found to work well with the ADIFAB2 probe. 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 ADIFAB ratio changes over time