

## Preparation and Use of ADIFAB2

**When you receive your order of ADIFAB2, do the next three steps IMMEDIATELY :**

- 1. FREEZE (at -70°C if possible) LYOPHOLIZED ADIFAB2**
- 2. FREEZE (at -20°C) STANDARD(S)**
- 3. REFRIGERATE (at 4°C) STORAGE AND MEASURING BUFFERS**

### **Preparation of ADIFAB2 stock solution:**

Add storage buffer to the vial of lyophilized ADIFAB2 powder to give a final concentration of ~100  $\mu\text{M}$ . The approximate molecular weight of ADIFAB2 is 15000 g/mole; therefore, for every 200  $\mu\text{g}$  ADIFAB2, add 133  $\mu\text{L}$  storage buffer and for 1 mg ADIFAB2, add 667  $\mu\text{L}$ . Once storage buffer is added to ADIFAB2, store at 4°C. The storage buffer consists of 20 mM HEPES, 140 mM NaCl, 5 mM KCL, 1 mM Na<sub>2</sub>HPO<sub>4</sub>, 1 mM EDTA and 0.05% sodium azide. The pH at room temperature is  $7.4 \pm 0.1$ .

### **Measuring Buffer:**

Measuring buffer consists of 20 mM HEPES, 140 mM NaCl, 5 mM KCl, and 1 mM Na<sub>2</sub>HPO<sub>4</sub>. The pH at room temperature equals  $7.4 \pm 0.1$ . Store the measuring buffer at 4°C. The recommended concentration of ADIFAB2 to be used in the measuring buffer is approximately 0.5  $\mu\text{M}$ . 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 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. 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 ADIFAB2 ratio changes over time