

## Preparation and Use of PAL and OAL

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

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

### Preparation of stock solution for the Probes:

The probe is lyophilized in measuring buffer plus 1 mM EDTA and 0.05% sodium azide. Simply resuspend the lyophilized probe in enough deionized/distilled (DI) water to give a final concentration of 100  $\mu$ M.

The approximate molecular weight of each probe is 15000 g/mole; therefore, for every 200  $\mu$ g probe, add 133  $\mu$ L DI water and for 1mg probe, add 667  $\mu$ L. Once the probe is solubilized, store at 4°C. The pH at room temperature is  $7.4 \pm 0.1$ .

### Measuring Buffer:

The 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 each probe to be used in the measuring buffer is approximately 0.5  $\mu$ 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 a measurement using one of the probes. 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# 67.741) have been found to work well with our probes. However acrylic cuvettes can leach a substance that reacts with the probe. Cuvettes made of other materials can be easily tested by determining if the probe ratio changes over time.

### Assay Method:

You can determine unbound free fatty acid levels in an aqueous solution with a simple, one step procedure: add a small amount of the reagent to a solution suspected of containing unbound free fatty acid and measure the resulting fluorescence. The excitation wavelength for the probes is 375 nm and the emission wavelengths are 550 and 457 nm.

### Characteristics

- **Sensitivity: less than 1 nM unbound palmitate**
- **Purity : Gel electrophoresis > 95%**
- **Form: Lyophilized powder**
- **Weight: 15000 Da**