

## SunRed™ Phosphate

Catalog number: 11629

Unit size: 5 mg

Component	Storage	Amount
SunRed™ Phosphate	Freeze (<-15 °C), Minimize light exposure	5 mg

### OVERVIEW

Phosphatase-catalyzed hydrolysis of Sun Red phosphate (SRP) yields the Sun Red fluorophore that can be excited with the 633 nm laser with emission of ~660 nm. Although Sun Red is readily excited at 633 nm with red emission of ~660 nm, SRP has very minimal absorption at 633 nm without red emission, making SRP one of the most sensitive NIR phosphatase sensors. Please do not use DMSO to make stock solution since it significantly increases assay background.

### AT A GLANCE

#### Protocol summary

1. Prepare and add 10 - 50  $\mu\text{M}$  SunRed™ Phosphate in Tris buffer (50  $\mu\text{L}$ )
2. Add alkaline phosphatase standards and/or test samples (50  $\mu\text{L}$ )
3. Incubate at room temperature or 37°C for 30 to 120 minutes
4. Monitor fluorescence intensity at Ex/Em = 620/660 nm (cut off 640 nm)

**Important** The following is the recommended protocol for alkaline phosphatase assay in solution. The protocol only provides a guideline, should be modified according to the specific needs.

### KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	620 nm
Emission:	660 nm
Cutoff:	640 nm
Recommended plate:	Solid black

### PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

#### 1. SunRed™ Phosphate stock solution:

Prepare a 2 to 10 mM stock solution of SunRed™ Phosphate in sterile water.

**Note** The stock solution should be used promptly.

**Note** Do not use DMSO, ETOH or METH to make stock solution since it significantly increases assay background.

**Note** Protect from light.

### PREPARATION OF WORKING SOLUTION

#### SunRed™ Phosphate working solution (2X):

On the day of the experiment, either dissolve SunRed™ Phosphate solid in sterile H<sub>2</sub>O or thaw an aliquot of the SunRed™ Phosphate stock solution at room temperature. Prepare a 2X working solution of 10 to 50  $\mu\text{M}$  in 100 mM Tris buffer or buffer of your choice, pH 8 to 9. SunRed™ Phosphate final concentration of 5 to 25  $\mu\text{M}$  is recommended for measuring alkaline phosphatase activity in solution.

### SAMPLE EXPERIMENTAL PROTOCOL

1. Add 50  $\mu\text{L}$  of 2X SunRed™ Phosphate working solution into each well of the alkaline phosphatase standard, blank control, and test samples to make the total alkaline phosphatase assay volume of 100  $\mu\text{L}$ /well. For a 384-well plate, add 25  $\mu\text{L}$  of sample and 25  $\mu\text{L}$  of 2X SunRed™ Phosphate working solution into

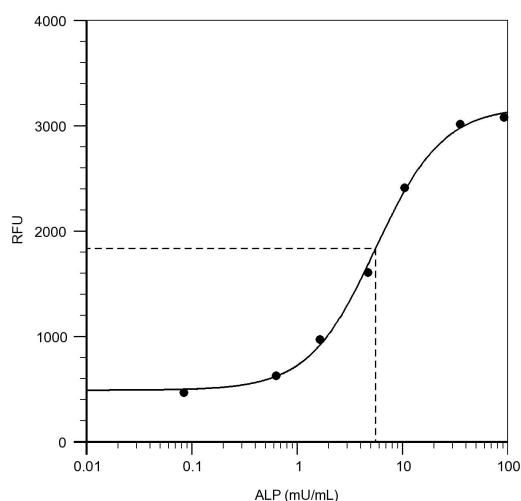
each well.

2. Incubate the reaction for 30 to 120 minutes at the desired temperature, protected from light.
3. Monitor the fluorescence increase at Ex/Em = 620/660 nm (cut off at 640 nm) with a fluorescence plate reader.

### EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the baseline corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate ALP samples. We recommend using the Online Four Parameter Logistics Calculator which can be found at:

<https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator>



**Figure 1.** Alkaline phosphatase dose response was measured with the Amplitude™ Fluorimetric Alkaline Phosphatase Assay Kit in a solid black 96-well plate using a Gemini microplate reader (Molecular Devices).

### DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email [info@aatbio.com](mailto:info@aatbio.com) if you have any questions.