Alkaline and Acid Phasphatase Substrate: FDP, MUP, CF-MUP, and SunRed™ Phosphate

Introduction

Upon interaction with phosphatases the colorless and non-fluorescent FDP is hydrolyzed to highly fluorescent fluorescein, which exhibits excellent spectral properties that match the optimal detection window of most fluorescence instruments that are equipped with the Argon laser excitation. Alternatively, FDP can also be used to detect phosphatases in a chromogenic mode since the enzymatic product (fluorescein) exhibits a large extinction coefficient (close to 100,000 cm-1mol-1). In some literature, FDP was considered to be one of the most sensitive fluorogenic phosphatase substrates. FDP has been widely used in various ELISA assays. Additionally it is also used to detect tyrosine phosphatases. FDP is thermally unstable, and special cautions need be excised for storing the solid sample and stock solutions.

Mup is A fluorogenic substrate for phosphatases. It is widely used for detecting phosphatases in solution. However this phosphatase substrate is not well suited for living cell or continuous assays since MU (4-methylumbelliferone), the enzymatic product, which only develops maximum fluorescence at pH value of >10. Thus it is also difficult to use MUP for the detection of phosphatases that have acidic optimal pH range such as acid phosphatases.

AAT Bioquest is pleased to offer CF-MUP Plus that is developed to address this pH limitation associated with MUP substrates. CF-MU exhibits maximum fluorescence above pH 5.0, thus CF-MUP substrate can be well used for continuous phosphatase assays. It can also be used for the assays that require acidic pH such as acid phosphatases and some protein phosphatases.

The phosphatase substrate derived from the red-fluorescent dimethylacridinone (Sun Red) contains only a single hydrolysis sensitive moiety, thereby avoiding the biphasic kinetics of fluorescein-based substrates. 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.

Chemical and Physical Properties

Product number	Substrate	Size	MW	Ex/Em (nm)	pKa	Solvent	Phosphatases Detected
11600	FDP	5 mg	560.39	490/514	6.4 **	ddH2O	Protein tyrosine phosphatases [2,3] Alkaline phosphatase [4,5]
11610 11612	MUP, Na Salt	25 mg 10g	300.11	360/449	7.8 §	ddH2O	Alkaline phosphatase [6,7]
11614 11617	MUP, free acid	25 mg 10g	256.15	30/449	7.8 §	DMSO	Alkaline phosphatase [6,7]
11627	DiFMUP	5 mg	292.13	360/450	4.7	DMSO	Acid phosphatase 8 Alkaline phosphatase [6,7] Protein phosphatase 1 (PP1) [9,10] Protein phosphatase 2A (PP2A) [9,10]
11629	SunRed™ Phosphate	5 mg	422.20	646/659	~6.0	ddH2O*	Protein phosphatase 2A (PP2A) [1]

** Phosphatase substrates can be detected by phosphatases that may not be specifically listed. . Toxicon 38, 1833 (2000). § Bioorg Med Chem Lett 8, 3107 (1998). 1. Toxicon 38, 1833 (2000); 2. Biochem J 337, 219 (1999); 3. Biochim Byophys Acta 1431, 14 (1999); 4. Nature Biotech 18, 847 (2000); 5. Science 283, 1892 (1999); 6. J Microbiol Methods 40, 147 (2000); 7. Micron 31, 41 (2000); 8. *Current Chemical Genomics*, 2008, *2*, 48-50. 9. Anal Biochem 248, 258 (1997); 10. Anal Biochem 269, 289 (1999).

* Do not use DMSO to make SunRed™ Phosphate stock solution

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Assay Protocol with Phosphate Substrate

Brief Summary

Prepare 10-50 µM Phosphate in Tris buffer (50 µL) → Add phosphatase standards and/or test samples (50 µL) → Incubate at RT or 37 °C for 30 to 120 minutes → Monitor fluorescence intensity

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

1. Prepare working solution:

1.1 Prepare a 2 to 10 mM stock solution in an appreciate solvent (see Table). The stock solution should be used promptly. Any unused solution need to be aliquoted and frozen at ≤ -20 °C.
*Note1: **Do not use DMSO, ETOH or METH** to make stock solution of SunRed™ Phosphate (cat#11629) since it significantly increases assay background.*
Note2: Avoid repeated freeze-thaw cycles, and protect from light.

1.2 Prepare a 2X Phosphate working solution: On the day of the experiment, either dissolves the substrate in an appreciate solvent or thaw an aliquot of the stock solution (from Step 1.1) at room temperature. Prepare a 2X working solution of 10 to 50 µM in 100 mM Tris buffer or buffer of your choice, pH 8 to 9 (not phosphate buffer).

2. Run phosphatase assay in supernatants:

2.1 Add 50 µL of 2X Phosphate working solution (from Step 1.2) into each well of the phosphatase standard, blank control, and test samples to make the total phosphatase assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of 2X Phosphate working solution into each well.

2.2 Incubate the reaction for 30 to 120 minutes at the desired temperature, protected from light.

2.3 Monitor the fluorescence increase at an appropriate filter sets (See table) with a fluorescence plate reader.

2.4 The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the phosphatase reactions.