

**FDP [Fluorescein diphosphate, tetraammonium salt] \*CAS 217305-49-2\***

Catalog number: 11600

Unit size: 5 mg

Component	Storage	Amount
FDP [Fluorescein diphosphate, tetraammonium salt] *CAS 217305-49-2*	Freeze (<-15 °C), Minimize light exposure	5 mg

**OVERVIEW**

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<sup>-1</sup>mol<sup>-1</sup>). 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 to be exercised for storing the solid sample and stock solutions.

**AT A GLANCE**
**Protocol summary**

1. Prepare 10 - 50 μM Phosphate in Tris buffer (50 μL)
2. Add phosphatase standards and/or test samples
3. Incubate at room temperature or 37°C for 30 to 120 minutes
4. Monitor fluorescence intensity at Ex/Em= 490/514 nm

**Important** Phosphatase substrates can be detected by phosphatases that may not be specifically listed. 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.

**KEY PARAMETERS**

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	514 nm
Cutoff:	500 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. FDP stock solution:**

Prepare a 2 to 10 mM stock solution in ddH<sub>2</sub>O.

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

**PREPARATION OF WORKING SOLUTION**
**FDP working solution (2X):**

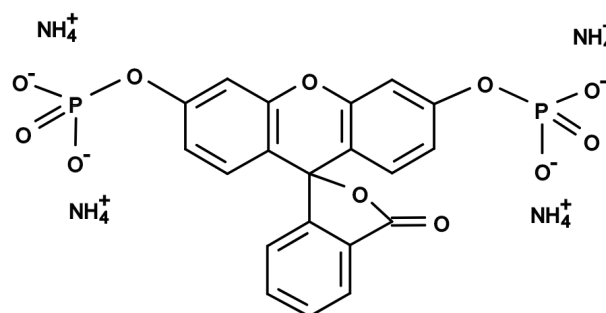
On the day of the experiment, either dissolve FDP in ddH<sub>2</sub>O or thaw an aliquot of the stock solution at room temperature. Prepare a 2X working solution of 10 to 50 μM in 100 mM Tris buffer or buffer of your choice, pH 8 to 9 (not phosphate buffer).

**SAMPLE EXPERIMENTAL PROTOCOL**

1. Add 50 μL of 2X FDP working solution into each well of the phosphatase standard, blank control, and test samples to make the total phosphatase assay volume of 100 μL/well. For a 384-well plate, add 25 μL of sample and 25 μL of

2X 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 an appropriate filter set with a fluorescence plate reader.
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.

**EXAMPLE DATA ANALYSIS AND FIGURES**


**Figure 1.** Chemical structure for FDP [Fluorescein diphosphate, tetraammonium salt] \*CAS 217305-49-2\*

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