

For life science research only.  
Not for use in diagnostic procedures.



# AttoPhos Substrate Set

 **Version: 11**

Content Version: December 2020

Fluorescent alkaline phosphatase substrate to be used in microplate format assays.

**Cat. No. 11 681 982 001**    1 set  
1,800 wells, 100 µl each or 720 tubes

**Store the product at +2 to +8°C.**

<b>1.</b>	<b>General Information .....</b>	<b>3</b>
1.1.	Contents .....	3
1.2.	Storage and Stability .....	3
	Storage Conditions (Product) .....	3
1.3.	Additional Equipment and Reagent required .....	3
1.4.	Application .....	3
	Product Description .....	3
<b>2.</b>	<b>How to Use this Product .....</b>	<b>4</b>
2.1.	Before you Begin .....	4
	General Considerations .....	4
	Specifications .....	4
	AttoPhos substrate .....	4
	Calibrator solution .....	4
	Safety Information .....	5
	AttoPhos substrate .....	5
	AttoPhos buffer .....	5
	Waste disposal .....	5
	Laboratory procedures .....	5
	Waste handling .....	5
	Working Solution .....	5
2.2.	Protocols .....	6
	ELISA .....	6
2.3.	Parameters .....	6
	Sensitivity .....	6
<b>3.</b>	<b>Additional Information on this Product .....</b>	<b>6</b>
3.1.	Test Principle .....	6
3.2.	Quality Control .....	6
<b>4.</b>	<b>Supplementary Information .....</b>	<b>7</b>
4.1.	Conventions .....	7
4.2.	Changes to previous version .....	7
4.3.	Trademarks .....	8
4.4.	License Disclaimer .....	8
4.5.	Regulatory Disclaimer .....	8
4.6.	Safety Data Sheet .....	8
4.7.	Contact and Support .....	8

# 1. General Information

## 1.1. Contents

Vial / bottle	Label	Function / description	Content
1	AttoPhos Substrate Set, AttoPhos substrate	Each bottle contains 36 mg of AttoPhos substrate.	3 bottles, 60 ml each
2	AttoPhos Substrate Set, AttoPhos buffer	Ultra-pure aqueous buffer solution of 2.4 M diethanolamine, 0.057 mM MgCl <sub>2</sub> , 0.005% NaN <sub>3</sub> (w/v), pH 10.0.	4 bottles, 60 ml each
3	AttoPhos Substrate Set, Calibrator solution	1.7 M solution of pure fluorescent emitter (500 ng/ml) in 2.4 M diethanolamine, 0.005% NaN <sub>3</sub> (w/v), pH 10.0.	1 bottle, 5 ml

## 1.2. Storage and Stability

### Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	AttoPhos substrate	Store at +2 to +8°C. ⚠ <b>Keep protected from light and oxidizing agents.</b>
2	AttoPhos buffer	Store at +2 to +8°C.
3	Calibrator solution	Store at +2 to +8°C. ⚠ <b>Keep protected from light and oxidizing agents.</b>

## 1.3. Additional Equipment and Reagent required

### For ELISA

- Fluorometer
- Transparent or black microplates

## 1.4. Application

Use the AttoPhos Substrate Set as a substrate for enzyme-linked immunosorbent assays (ELISA) using alkaline phosphatase-conjugated secondary detection systems.

### Product Description

AttoPhos is a highly sensitive fluorimetric substrate for the detection of alkaline phosphatase. With an optimal excitation wavelength of 430 to 440 nm and the emission maximum at 560 nm, an exceptionally large Stokes Shift is seen. This results in an extremely low intrinsic background signal with a detection limit of 10<sup>-19</sup> moles for alkaline phosphatase. In addition, the background fluorescence caused by biological molecules (assay components), cuvettes, or microplates is greatly reduced. With these unique properties, AttoPhos is more sensitive by a factor of 100 than the most commonly used fluorescence substrate 4-methylumbelliferyl-phosphate (MUP).

## 2. How to Use this Product

### 2.1. Before you Begin

#### General Considerations

##### Specifications

Many of the unique features of AttoPhos result from the excitation and emission wavelengths utilized with the substrate. The large Stokes Shift of 140 nm is the major contributor to the extremely low background emission, and consequently the exceptionally low detection limits of  $10^{-19}$  moles of alkaline phosphatase. From the specifications described, it is clear that extreme care must be taken at all times to avoid alkaline phosphatase contamination from all sources in order to benefit from the low background emission that is inherent to AttoPhos.

##### AttoPhos substrate

Parameter	Value
Molecular weight	Approximately 580 g/mol
Solubility	$\geq 10$ mM in 2.4 M diethanolamine buffer, pH 9.0
Optimal concentration	0.5 to 1.5 mM with alkaline phosphatase
$K_m$	0.03 mM
Turnover number	98,200 molecules/minute in 2.4 M diethanolamine, 0.23 mM $MgCl_2$ , 0.005% $NaN_3$ (w/v), pH 9.0
Molar absorptivity	31,412

##### Calibrator solution

Parameter	Value
Molecular weight	Approximately 290 g/mol
UV maximum	418 nm with a molar extinction coefficient of 26,484 in 0.392 M $Na_2CO_3$ , pH 11
Excitation maximum	420 nm
Emission maximum	560 nm
Stokes shift	140 nm
Water Raman	Emission occurs at 478 nm with an excitation of 413 nm. There is no overlap of the water Raman emission of the fluorescent emitter at 560 nm.
Optimum excitation/ emission wavelength	440 nm with monitoring at 550 nm when using DEA buffer and a fluorometer. <ul style="list-style-type: none"><li>▪ Optimum operating parameters may vary with fluorometers from other manufacturers.</li><li>▪ Determine the optimum conditions for excitation and emission on other manufacturers' fluorometers, or contact the instrument manufacturer to see if optimized filter sets exist.</li></ul>

## Safety Information

### AttoPhos substrate

- Avoid contact and inhalation.
- Toxicological properties of this material have not been investigated; exercise due care.

### AttoPhos buffer

- Avoid contact and inhalation.
- If contact with eyes, immediately flush with large amounts of water for at least 15 minutes.
- If contact with skin, immediately wash skin with soap and large amounts of water.

### Waste disposal

Dispose of waste according to local regulations.

### Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

### Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on [dialog.roche.com](http://dialog.roche.com), or upon request from the local Roche office.

## Working Solution

Solution	Preparation/Composition	Storage and Stability
AttoPhos working solution	Transfer the entire content of one bottle AttoPhos buffer (Bottle 2) into one bottle AttoPhos substrate (Bottle 1). <b>i</b> <i>The resulting AttoPhos substrate concentration is 1 mM.</i>	Store 2 weeks at +2 to +8°C or at least 6 months at –15 to –25°C. Mix thoroughly to avoid inhomogeneity after thawing.
Calibrator solution	Prepare appropriate dilutions of Calibrator stock solution (Bottle 3) using AttoPhos buffer solution (bottle 2).	Store for at least 6 months at +2 to +8°C.

### 3. Additional Information on this Product

## 2.2. Protocols

### ELISA

Perform ELISA using transparent or black microplates.

**⚠** *White plates from different manufacturers showed high levels of auto-fluorescence at  $ex_{444\text{ nm}}/em_{555\text{ nm}}$ .*

**i** See section, **Working Solution**, for information on preparing solutions.

- 1 Wash microplate carefully to reduce background of nonspecifically bound alkaline phosphatase.

---

- 2 Depending on the volumes used for coating and antibody reaction, apply 50 to 250  $\mu\text{l}$  of AttoPhos working solution.

---

- 3 Allow the alkaline phosphatase reaction to proceed at +20 to +37°C for 5 to 60 minutes prior to measurement.  
– Depending on the assay background, even longer incubation times may improve sensitivity. Repeated measurements are possible.

---

- 4 Optimal properties with respect to signal-to-noise ratio can be obtained at an excitation wavelength of approximately 440 nm and an emission wavelength of approximately 550 nm.

---

## 2.3. Parameters

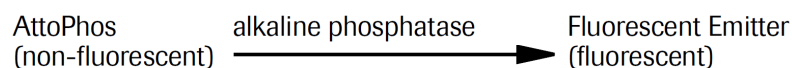
### Sensitivity

Depending on the affinity of the primary antibody, 1 to 5 pg amounts of antigen can be detected.

## 3. Additional Information on this Product

### 3.1. Test Principle

The formation of the fluorochrome (fluorescent emitter) occurs when an alkaline phosphatase-labeled antibody, probe, or other entity liberates the phosphate group from the non-fluorescent AttoPhos molecule. The simple one-step reaction mechanism exhibits excellent linear kinetics over three decades of alkaline phosphatase concentration.



### 3.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

## 4. Supplementary Information

### 4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

#### Text convention and symbols

 *Information Note: Additional information about the current topic or procedure.*

 **Important Note: Information critical to the success of the current procedure or use of the product.**

① ② ③ etc. Stages in a process that usually occur in the order listed.

① ② ③ etc. Steps in a procedure that must be performed in the order listed.

\* (Asterisk) The Asterisk denotes a product available from Roche Diagnostics.

### 4.2. Changes to previous version

Layout changes.

Editorial changes.

Update to include new safety Information to ensure handling according controlled conditions.

## 4. Supplementary Information

### 4.3. Trademarks

All product names and trademarks are the property of their respective owners.

### 4.4. License Disclaimer

For patent license limitations for individual products please refer to:

**List of biochemical reagent products.**

### 4.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

### 4.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

### 4.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site.**

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

