

# FLUOROPHOS®

## Alkaline Phosphatase (ALP) Reagent Set

FLA224/FLA225

FLA224      Two (2) bottles of Substrate Buffer (2 x 240 mL)  
Two (2) bottles of Substrate (2 x 144 mg)

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Fluorophos Cuvettes - 250/pack (p/n FLA020)

### Intended use:

The Fluorophos ALP assay is intended for use with the Advanced Instruments Fluorophos ALP Test System fluorometer for the quantitative determination of alkaline phosphatase (ALP) activity in dairy products.

### Summary and principle of the assay:

The Fluorophos ALP assay is based on a quantitative procedure using a benzothiazole phosphate derivative as substrate. The ALP activity of the sample is measured by a continuous fluorimetric direct kinetic assay.

The Fluorophos ALP substrate 2'-[2-benzothiazoyl]-6'-hydroxybenzothiazole phosphate—in the presence of any alkaline phosphatase derived from the sample—undergoes hydrolysis of its phosphate radical, producing a highly-fluorescent product 2'-[2-benzothiazoyl]-6'-hydroxybenzothiazole (Fluoroyellow®). The Fluoroyellow is monitored in a real-time kinetic mode on the Advanced Instruments fluorometer. Excitation and emission are in the visible light range at 440 nm (blue) and 530 nm (yellow), respectively.

The Fluorophos ALP method is a three-minute test. The first minute of the assay is used to pre-incubate the substrate and sample mixture in the fluorometer to ensure a reaction temperature of  $38 \pm 0.2^\circ\text{C}$ . During minutes two and three, the reaction is continuously monitored and the increase in fluorescence is recorded.

### Ingredients:

Fluorophos substrate	2'-[2-benzothiazoyl]-6'-hydroxybenzothiazole phosphate	144 mg
Substrate buffer	2.4 M Diethanolamine (DEA) pH 10.0	240 mL

### Warning:

Reagents are harmful when inhaled, when swallowed, and when they come into contact with skin. Reagents pose danger of serious damage to health by prolonged exposure if swallowed. Refer to Safety Data Sheet SDS019 at <https://www.aicompanies.com/support/safety-data-sheet/> for complete hazard information.

### Instructions for use:

#### Preparing the reagent

1. Allow one bottle of Fluorophos substrate and one bottle of substrate buffer to come to room temperature.
2. To reconstitute, carefully decant the entire contents of the substrate buffer bottle into the Fluorophos substrate bottle.
3. Mix by gentle inversion and allow the mixture to sit at room temperature for at least thirty minutes, or until the Fluorophos substrate is fully dissolved.

## Notes

- Do not mix bottles from different reagent sets.
- Do not use stir bars or mechanical stirring devices.
- Do not put pipette tips or other dispensing devices directly into the bottle. Instead, pour the desired volume into a separate, clean container for pipetting into disposable cuvettes.

## Sample preparation

Refer to the *Sample Preparation* section of the *Fluorophos ALP Test System User's Guide* (p/n FL3005EN).

## Test procedure

For instructions using the Daily Instrument Control (FLA280) and Calibrator Set (FLA250), see the *Fluorophos Test System ALP User's Guide* and product inserts (<https://www.aicompanies.com/support/>).

## Notes

- To make sure that the main prepared bottle is safe from contamination, pour off the amount needed for testing and pipette from that amount.
- Use a new pipette tip with each string of pipetting.
- Start the test within 20 seconds after adding the test sample to the reagent.
- Do not allow the sample to sit in the heating block once it has been added to the reagent.
- Prepare and test only one sample at a time.

## Storage and handling:

	Storage	Stability
Unopened	2-8 °C (36-46 °F); protect from light	Two (2) years
Reconstituted	2-8 °C (36-46 °F); protect from light	Sixty (60) days
In the heating block	38 ±1.0 °C	Six (6) hours

## Limitations:

Erroneous results can occur from adverse shipping and/or storage conditions, use of expired materials, sample handling errors.

## Interpretation of results:

Any sample that yields a test result under 350 mμ/L is considered ALP negative, indicating proper and complete pasteurization. Any sample that yields a test result over 350 mμ/L is considered ALP positive for dairy, reactivated and/or microbial ALP. Refer to the *Fluorophos ALP Test System User's Guide* for additional testing of ALP positive samples.

## Assay performance

The assay is linear to at least 2000 mμ/L.

## Disposing of materials:

Handle this product according to established good laboratory practices, using appropriate precautions. Dispose materials according to your institution's practices. Discard all materials in a safe and acceptable manner that is in compliance with all country, state and local requirements.



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