

# New Bioluminescent Alkaline Phosphatase Test for Verification of Milk Pasteurization



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## Introduction:

Alkaline phosphatase has been used to verify dairy pasteurization since 1935. This study introduces a new technology from Hygiena for the monitoring of alkaline phosphatase verification from pasteurised milk samples that utilizes new bioluminescent substrates in a simple self-contained device to measure and interpret results from samples in a new luminometer. The test has been validated against milk in UK, USA and India.

## Purpose:

This study demonstrates the performance of ZymoSnap ALP as a rapid and efficient method for dairy pasteurization verification.

## Methods:

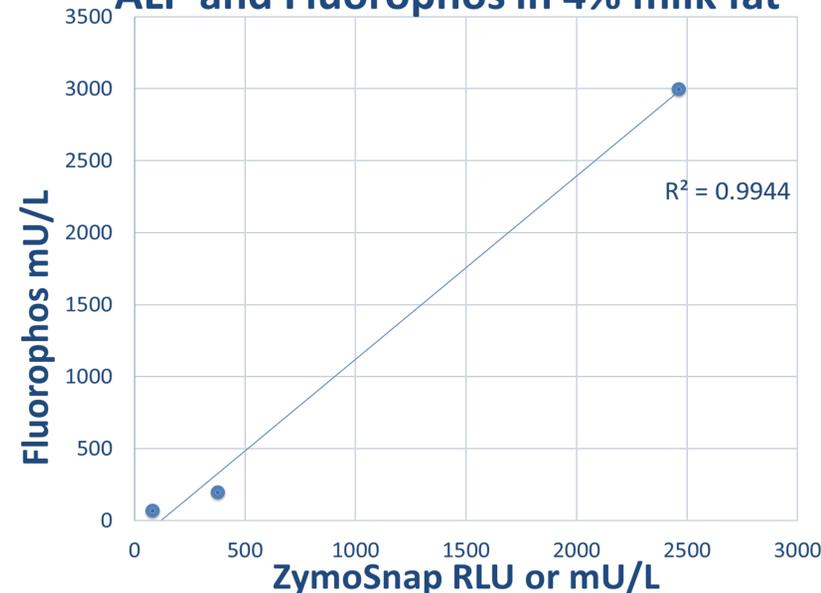
Pasteurized milks containing 0.1%, 2%, and 4% fat solids were obtained from 10 different dairy brands for evaluation in two assays with various Hygiena luminometers. Both assays produce equivalent results to the “gold standard,” Fluorophos system. Each milk sample was spiked with 4 levels of bovine alkaline phosphatase to the following levels 0 mU/L, 100 mU/L, 350 mU/L and 1000 mU/L. Each milk was assayed using 5 replicates with means compared and charted for correlation.

Table 1. Comparison of Fluorophos vs. ZymoSnap ALP

Fluorophos	1000 mU/L	350 mU/L	100 mU/L	0 mU/L
4% milk	2462.7	377.3	82.2	<10
2% milk	2252.5	227.8	73.2	<10
0.1% milk	2135.6	167.0	40.9	<10

Zymosnap ALP	1000 mU/L	350 mU/L	100 mU/L	0 mU/L
4% milk	2997.0	394.8	66.2	10.6
2% milk	3473.2	294.6	96.4	10.2
0.1% milk	6470.1	561.2	182.4	9.2

Correlation of Hygiena ZymoSnap ALP and Fluorophos in 4% milk fat



## Results:

The assay demonstrated excellent linearity at all milk fat contents and demonstrated an inversely proportional relationship between fat content and light output. The correlation coefficients for the 10 milk types at n=5 was found to be greater than  $R^2 = 0.990$ . The conversion of RLU (relative light unit) to mU/L of alkaline phosphatase was found to be most effected by fat content of the milk which resulted in the following conversion factors: 4% RLU=1 mU/L, at 2% RLU=0.7 mU/L, and at 0.1% RLU=0.3 mU/L., respectively.

## Significance:

Zymosnap ALP, a new technology from Hygiena, has demonstrated rapid and efficient measure of alkaline phosphatase for verification of milk pasteurization methods. There was found to be a strong correlation of ALP detection between test methods in pasteurized milk. Future analysis will investigate the potential of success for Zymosnap ALP to be adapted to more complex dairy matrices such as flavored milks, yogurts, and cheeses.

REFERENCES:  
 1. Kay, H.D. and Graham, W.R., The phosphatase test for pasteurized milk. J. Dairy Res. 6:191-203 (1935).  
 2. Kim, Dong-Hyeon et al. "Establishing Quantitative Standards for Residual Alkaline Phosphatase in Pasteurized Milk." Korean Journal for Food Science of Animal Resources 36.2 (2016): 194-197. PMC. Web. 30 Aug. 2017.