

Alkaline Phosphatase Test Strips

Description and intended use

Alkaline Phosphatase (ALP) is a heat sensitive enzyme found naturally in raw milk and is used in the dairy industry as an indicator of proper milk pasteurization. Complete pasteurization will inactivate the enzyme to below levels which are detectable by conventional methods. Because the heat stability of ALP is greater than that of pathogens which may be present in milk, the enzyme serves as an indicator of product safety.

However, the failure to detect ALP activity does not guarantee that the product is pathogen free and other microbial test such as coliforms will be a better indicator of cross contamination post pasteurization.

The current ALP rapid test kit is based on chemical reaction of ALP with its substrate to detect ALP activity in pasteurized milk. It is measured as Milli Units per Litre (mU/L) of enzyme and the cut-off level is 100mU/L which is 2.0 p-nitrophenol (Ug/mL) and therefore meets the minimum level of <10 p-nitrophenol (Ug/mL).

The assay can confirm whether milk samples meet the required safe level of Alkaline Phosphatase in 60 minutes at 37°C.

Principle

The assay uses a receptor strip which milk is carefully added to the strip and incubated at 37°C for 60 minutes or room temperature for 90 minutes. The test is sensitive enough to measure below the 100mU/L of ALP or <2.0 p-nitrophenol (Ug/mL).

Test Procedure

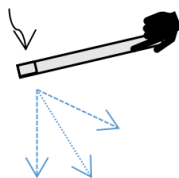
- Bring the test kit and samples to room temperature.
- Immerse the reaction zone of the test strip into the test sample (15 – 30°C) for 1 s.
- Seal the cap of the bottles and store the unneeded kit.
- Shake off excess liquid from the strip.
- Seal it in a PE bag to avoid dryness of the test strip and place into small bag during incubation. Alternatively, you can insert the bag into the small tubes and incubate using the MiniS incubator (dry block heater).
- Incubate the sealed test strip at 37°C for 60 minutes
- Then take out and observe the result.



Sample



Dip



Shake



Seal



37 °C 60min

Incubation

Interpretation of Results

Positive: the test zone on the strip is yellow or light yellow. The ALP activity is over 100mU/L or more than 0.01% presence of raw milk.

Negative: the test zone remains no color. The ALP activity is less than 100mU/L. Detection limit: 0.5 % raw milk in pasteurized milk, 100mU/L ALP in pasteurized milk.



Refer to Table 1 for more interpretation of the results.

Table 1: Alkaline Phosphatase mU/L is directly proportional to the % of raw milk.

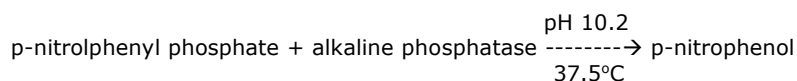
Alkaline Phosphatase mU/L	Alkaline Phosphatase (Ug/mL*)	% Raw Milk
100	2.0	0.01
350	7.0	0.05
500	10.0	0.10
1000	20.0	0.15

A level of 100 mU/L of Alkaline Phosphatase is considered safe and is equivalent to 2.0 Ug/ml or <2.0 Ug/mL p-nitrophenol).

Table 2: NSW Dairy Corporation 2001: (B2.1: Aschaffenburg-Mullen Method) Page 3

Reading (Ug/mL)*	Interpretation	Pass/ Fail
0 – 10	Properly pasteurized	Pass
10 – 18	Slightly pasteurized	Fail
18 – 42	Underpasteurised	Fail
42 or greater	Grossly unpasteurised	Fail

* p-nitrophenol (Ug/mL) based on the following reaction



NSW Dairy Corporation Requirements: All results must be less than 10 Ug/mL to be considered safe.

References:

1. AS2300.1.10-2008: General methods and principles – Determination of phosphatase activity.
2. AS3993-2003: Equipment or the pasteurization of milk and other liquid dairy products – continuous-flow systems
3. NSW Dairy Corporation: Dairy Test Manual (Sep 2001): B2.1 Phosphatase Test (Aschaffenburg-Mullen Method)
4. Marshall, R.T (1992), Chapter 14, Alkaline Phosphatase Methods page 413 - 431, "Standard Methods for the Examination of Dairy Products"
5. NSW Food Authority – Version 2: Pasteurisation requirements