FMCG Industry Solutions Pty Ltd

ZymoSnap Alkaline Phosphatase V5

Description and intended use

The ZymoSnap Alkaline Phosphatase device is used for measuring the level of Alkaline Phosphatase (ALP) enzyme in milk and other milk based products. It is a rapid bioluminogenic method for determination of acceptable levels of ALP enzyme via the conversion of RLU (Relative Light Units) to Milli Units per Litre (MU/L) of enzyme, after pasteurization of milk. The assay can confirm whether or not milk samples meet the required safe level of ALP in 5 minutes.

Principle

The assay uses a single self-contained device in a simple procedure. Milk (75ul) is carefully added to the ZymoSnap ALP device tube, the device is activated by breaking the bulb to release the detection reagent into the tube and incubating it for 5 minutes. The enzymatic action on the substrates is measured via bioluminogenesis on the Hygiena Ensure Luminometer. The test is sensitive enough to measure below the 100mU/L of ALP or less than 2ug/mL.

Test Procedure

1. Allow test devices to equilibrate to room temperature (10 minutes). Lift the cap out of the tube. Pipette 75ul directly into the tube (the loop can also be used)
2. Carefully place the cap back onto the device tube.
3. Activate device by breaking the snap valve pin and squeezing the bulb to release the detection reagent into the tube.
4. Shake the tube gently for 5-10 seconds to mix reagent with sample liquid.
5. Incubate for 5 minutes ± 10 seconds at 37°C ± 1°C.
6. During incubation time, turn on luminometer. If luminometer has been programmed, select appropriate program.
7. Immediately place entire device into the Hygenia Ensure luminometer and press "OK" to read RLUs. Results will be displayed in 15 seconds.
8. Positive Control: 0.2ml fresh raw milk to 100mL of milk heated to 95°C for 1 minute.
9. Negative control: 5ml of milk heated to 95°C for 1 minute.
10. Microbial Alkaline Phosphatase Control: If the sample produces a positive results then proceed as follows: Take another test portion, heat to 62.8°C, hold it at this temperature for 30 minutes and then cool rapidly. Repeat test.
11. Results are tabulated in Table 1.
12. A result of less than 350 RLU (Full Cream Milk), 525 RLU (2% Milk) or 1000 RLU (Skim Milk) for the ZymoSnap ALP is considered safe and is equivalent to less than 7 ug/mL of p-nitrophenol using Australian Standards AS2300.1.10-2008.

Interpretation of Results

Table 1: RLU displayed on the luminator is directly proportional to the mU/L of ALP

Alkaline Phosphatase mU/L	Milk RLU	2% Milk RLU	Skim Milk RLU	Alkaline Phosphatase Ug/mL*	% Raw Milk
100	100	150	300	2.0	0.01
350	350	525	1000	7.0	0.05
500	500	750	1500	10.0	0.10
1000	1000	1500	3000	20.0	0.15

A RLU of less than 350 for full cream milk, 525 for 2% milk and 1000 for skim milk on the Ensure is considered safe and is equivalent to <350mU/mL (UK and EU requirements or <7.0 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

$$\text{p-nitrolphenyl phosphate} + \text{alkaline phosphatase} \xrightarrow[37.5^{o}C]{\text{pH 10.2}} \text{p-nitrophenol}$$

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. ZymoSnap Alkaline Phosphatase – Catalog # ZSALP100: insert INS0105 Nov 16
5. Marshall, R.T (1992), Chapter 14, Alkaline Phosphatase Methods page 413 - 431, "Standard Methods for the Examination of Dairy Products"
6. NSW Food Authority – Version 2: Pasteurisation requirements