ALP Test Swab

USER MANUAL

Part Number: ALP0401

1. Description and Intended Use

ALP Test Swab measures Alkaline Phosphatase (ALP) activity in milk and other dairy products. The 5-minute test verifies pasteurization efficiency to meet regulatory standards and demonstrates the absence of raw milk. It is a rapid bioluminogenic method that determines the level of ALP enzyme via the conversion of Relative Light Units (RLUs) to milliunits per liter (mU/L) of ALP in pasteurized milk samples. ALP Test Swab is intended for use with pasteurized liquid milk and dairy products.

WARNING:

- This test swab is NOT applicable to UHT long-shelf-life milk and ambient-temperature stable dairy products.
- Testing these matrices will generate invalid results.

2. Principle

ALP test swab is designed for detecting Alkaline Phosphatase (ALP) residual in milk and dairy products through chemiluminescent reaction. Residual ALP in the sample catalyzes the hydrolysis of the phosphate group from the chemiluminescent substrate. Subsequent spontaneous molecular decomposition generates luminescent signals. The emitted light can be detected, and measured RLU values positively correlate with ALP activity, allowing quantitative detection by the ATP3500S Luminometer.

3. Required Materials (Not Provided)

a. ATP3500S Luminometer

b. Dry block Incubator or water bath, set at 37 ± 1°C

c. Water bath, set at 95 ± 1 °C (for preparation of Negative Control)

d. Vortex mixer

e. Timer

f. ALP0401E ALP (Alkaline Phosphatase) Positive Control Kit

g. Pipettes 10 µL – 100 µL and 100 µL – 1000 µL

h. Centrifuge tube 50 mL

i. Ice bath for cooling

4. Product Composition

Composition	Quantity
ALP Test Swab	100 swabs
ALP Positive Control	5 bottles
User manual	1 copy
COA	1 copy

5. Detection Sensitivity

ALP：20 mU/L (equivalent to approximately 0.002% of the typical ALP activity level found in raw milk)

6. Test Procedure

6.1 Pre-test Preparation

Before testing, take out the alkaline phosphatase (ALP) test swabs and equilibrate them to room temperature (20 – 25°C) away from light for 30 minutes.

Prior to testing, activate the alkaline phosphatase module on the ATP3500S Luminometer.

Switch on the dry incubator and preheat it until it maintains a stable temperature of 37±1°C.

Prepare a timer and set a 5-minute countdown.

6.2 Calibration

Prior to using ALP test swabs, positive and negative controls should be run initially. Calibration using negative and positive controls should be performed regularly (e.g., once a week), although the instrument default setting is once a month. Recalibration is required whenever a new manufacturing lot of ALP Test Swabs is introduced or when testing a different type of milk.

6.2.1 Preparation of Negative Control

a. Heat 5 – 10 mL milk sample in a 50 mL centrifuge tube in a water bath set at 95 ± 1°C for 2 minutes. The water level in the water bath shall

fully cover the milk sample. Do not heat the sample in a microwave oven. If protein precipitation occurs during preparation, heat the sample at 75°C for 15 min as an alternative.

b. Cool rapidly on ice, cover, and store at 2–8°C for up to 48 hours. Rapid cooling and cold storage are critical to avoid reactivating ALP.

Negative Control can be stored frozen at -15°C for up to 2 months.

c. Negative Control must be tested before preparation of Positive Control.

d. Use the Negative Control to reconstitute the Positive Control vial for the corresponding milk type.

6.2.2 Negative Quality Control Test

a. Lift the Snap-Valve bulb out of the tube.

b. Pipette 75 µL of the Negative Control into the tube.

c. Replace the bulb in the device tube. Shake and flick the device in downward strokes to ensure all sample reaches the bottom of the tube.

d. Activate the device by bending the bulb forward and backward to break Snap-Valve. Squeeze the bulb twice to release the reagent into the tube. Shake the swab for 5 seconds to mix the sample and reagent.

e. Incubate for 5 minutes±10 seconds at 37±1°C. During the incubation time, turn on the ATP3500S Luminometer and enter the alkaline phosphatase test module. Select whole pasteurized milk or skim pasteurized milk according to the type of test sample.

f. After the timer expires, shake the swab for 5 seconds to mix the sample and reagent.

g. Immediately place the ALP Test Swab into ATP3500S Luminometer for testing.

h. If the negative control sample passes the test (0–20 mU/L), proceed to sample testing. If the test fails, repeat the analysis. If two retests still yield unacceptable results, prepare a new negative control sample.

6.2.3 Preparation of Positive Control

Negative Control must be tested before preparation of Positive Control. Use the Negative Control to reconstitute the Positive Control vial for the corresponding milk type.

a. To avoid confusion, label each positive control vial with the milk type to be tested.

b. Carefully remove the metal seal and cap.

c. Pipette x mL (refer to the label on the positive control vial for the reconstitution volume) of the prepared Negative Control into the ALP Positive Control vial.

d. Replace the cap and vortex vigorously for 60 seconds to mix contents until completely dissolved.

e. After dissolution, keep refrigerated or in an ice bath for at least 10 minutes. Mix vigorously before each use. Store the vial at refrigerated temperatures (2–8°C) for 48 h. Refrigerated temperature shall be maintained during use.

6.2.4 Positive Control Test

a. Lift the Snap-Valve bulb out of the tube.

b. Pipette 75 µL of ALP Positive Control into ALP Test Swab tube.

c. Replace the bulb in the device tube. Shake and flick the device in downward strokes to ensure all sample reaches the bottom of the tube.

d. Activate the device by bending the bulb forward and backward to break Snap-Valve. Squeeze the bulb twice to release the reagent into the tube. Shake the swab for 5 seconds to mix the sample and reagent.

e. Incubate for 5 minutes±10 seconds at 37±1°C. During the incubation time, turn on the ATP3500S Luminometer and enter the alkaline phosphatase test module. Select whole pasteurized milk or skim pasteurized milk according to the type of test sample.

f. After the timer expires, shake the swab for 5 seconds to mix the sample and reagent.

g. Immediately place the ALP Test Swab into ATP3500S Luminometer for testing.

h. Repeat for a total of 2 replicates. If the positive control sample passes the test (225–525 mU/L), proceed to sample testing. If the test fails, repeat the analysis. If two retests still yield unacceptable results, prepare a new positive control sample.

6.3 Test

a. Lift the Snap-Valve bulb out of the tube. Pipette 75 µL of sample into the tube.

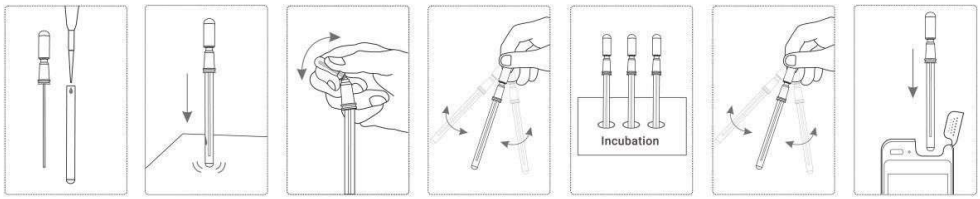
b. Replace the bulb in the device tube. Shake and flick the device in downward strokes to ensure all sample reaches the bottom of the tube.

c. Activate the device by bending the bulb forward and backward to break Snap-Valve. Squeeze the bulb twice to release the reagent into the tube. Shake the swab for 5 seconds to mix the sample and reagent.

d. Incubate for 5 minutes±10 seconds at 37±1°C. During the incubation time, turn on the ATP3500S Luminometer and enter the alkaline phosphatase test module.

e. After the timer expires, shake the swab for 5 seconds to mix the sample and reagent.

f. Immediately place the ALP Test Swab into ATP3500S Luminometer for testing.



7. Interpretation of Results

Table 1. Default RLU Threshold Settings of the Luminometer

Result	ATP3500S Luminometer (mU/L)	Interpretation and action
Pass	≤ 100 mU/L	Negative
Caution	100 – 350 mU/L	Confirmation shall be performed by microbiological methods
Fail[a]	≥ 350 mU/L	Positive

a.Where feasible, it is recommended to confirm whether the alkaline phosphatase is heat-stable or reactivated.

8. Testing Other Milk Products

This product has been validated for pasteurized milk samples. For testing other sample matrices, please consult Elaboric for technical support.

9. Storage and Shelf Life

2 – 8°C, protect from light, valid for 12 months.

10. Cautions and User Responsibility

ALP Test Swabs do not pose any health risk when used correctly. Used devices should be disposed of in compliance with Good Laboratory Practice and local Health & Safety Regulations.

a. ALP Test Swabs are designed for single use. Do not reuse.

b. Do not use ALP Test Swabs after the expiration date.

c. When activating ALP Test Swabs, ensure that all liquid in the bulb is transferred to the tube.

d. Ensure accurate incubation temperature and time.

e. ALP Test Swabs are light-sensitive. Do not leave swabs out of the bag for long periods of time.

Supplementary Notes

Confirmatory Test Methods for Alkaline Phosphatase-Positive Samples

1. Heat-Stable Alkaline Phosphatase

(1) Transfer 1 mL of suspected positive sample and incubate in a water bath at 63°C for 30 min; stir or mix thoroughly every 10 min. If the fat content of the sample exceeds 10 %, heat the sample at 66 ± 1°C for 30 min. Afterwards, immediately cool the sample in an ice bath to 0 – 4°C for testing.

(2) Test the negative control and positive control samples following the procedures specified in the Calibration section of the test method.

(3) Analyze the heat-treated sample and the untreated suspected positive sample in accordance with the alkaline phosphatase testing procedures.

(4) If the test results of the heat-treated sample and the untreated suspected positive sample are comparable, with a difference less than 30 % of the result of the untreated suspected positive sample, the alkaline phosphatase positivity is deemed to be caused by heat-resistant microorganisms.

(5) If the test result of the heat-treated sample is lower than that of the untreated suspected positive sample by a margin exceeding 30 % of the untreated sample's reading, the alkaline phosphatase positivity is attributed to residual alkaline phosphatase from incomplete pasteurization or reactivated alkaline phosphatase. Additional testing shall be performed to verify the presence of reactivated alkaline phosphatase.

2. Reactivated Alkaline Phosphatase

(1) Pipette 5 mL of suspected positive sample into a test tube, add 0.1 mL of water, and label as "Blank Sample". Pipette another 5 mL of the same suspected positive sample into a separate test tube, add 0.1 mL magnesium acetate solution (preparation method see Note at the end), and label as "Test Sample". Cap the tubes tightly, mix well, and incubate in a water bath at 34 ± 1°C for 1 h. Immediately cool both samples in

an ice bath to 0 – 4°C for subsequent testing.

(2) Pipette 1 mL of the magnesium acetate-containing Test Sample into a test tube, dilute with 5 mL negative control sample and mix thoroughly to prepare a 1:6 diluted sample, labeled "Diluted Sample".

(3) Test the Blank Sample (without magnesium acetate) and Diluted Sample (with magnesium acetate) following the alkaline phosphatase testing procedures.

(4) If the reading of the Diluted Sample is higher than or within 30 % of the reading of the Blank Sample, the alkaline phosphatase positivity is confirmed to originate from reactivated alkaline phosphatase.

(5) If the reading of the Diluted Sample is lower than that of the Blank Sample by a difference greater than 30 % of the Blank Sample's value, the alkaline phosphatase positivity is caused by residual alkaline phosphatase resulting from incomplete pasteurization.

Note: Preparation of Magnesium Acetate Solution

Weigh 35.4 g magnesium acetate tetrahydrate (CAS: 142-72-3) and dissolve in 25 mL water. Gentle heating may be applied to facilitate dissolution. Cool the solution to room temperature and adjust the final volume to 100 mL. Store at 0 – 4°C with a shelf life of 1 year.

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