Positive Control Kit for ZymoSnap ALP (Alkaline Phosphatase)

Part No: ZS-ALP-PC (5 vials)

Description and Intended Use

The Positive Control Kit for ZymoSnap ALP (Alkaline Phosphatase) devices consists of 5 vials of one concentration of ALP (350 mU/L). This is the globally recognized regulatory maximum for ALP levels in pasteurized milk products. A Negative Control is made by heat-inactivating a sample of the milk type the user is calibrating. This provides a background RLU reading that can be used as a baseline. Each Positive Control vial is then reconstituted with the heat-inactivated milk.

The Positive Control confirms the functionality of test devices and determines a Fail RLU level for operational testing. ZymoSnap ALP is sensitive enough to measure at typical pasteurization levels below the 350 mU/L regulatory level.

A Positive and Negative Control should be run initially to establish the Fail RLU level and then monthly for confirmation. Controls should also be run with each new manufacturing lot of ZymoSnap ALP test devices. Each type of milk requires its own Fail RLU level to be determined independently.

Required Materials (Not Provided)

1. Incubator or water bath set at 72 °C (for preparation of Negative Control)
2. Incubator set at 37 ± 1 °C (for test procedure)
3. Ice bath for cooling
4. ZymoSnap ALP test devices (Part No. ZS-ALP-100)
5. EnSURE® Touch Monitoring System (Part No. ETOUCH) or EnSURE Monitoring System (Part No. ENSURE)
6. Pipette and tips for 75 µL and 1.0 mL liquid transfer

Test Procedure

I. Preparation of Negative Control:

1. Heat 10 mL milk sample in a 0.5" x 4.0" test tube in an incubator or water bath set at 72 °C for 10 minutes.
2. 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.
3. Negative Control can be stored frozen at -20 °C for up to 3 weeks.
4. Negative Control must be tested before preparation of Positive Control as outlined in Section III.
5. Negative Control should be used to reconstitute Positive Control vials for testing the corresponding milk type (4%, 2%, 1%, etc.).

II. Preparation of Positive Control:

1. To avoid confusion, label each ALP vial with the milk type to be tested.
2. Carefully remove the metal seal and cap.
3. Pipette 1.0 mL of Negative Control made from milk type to be tested (4%, 2%, 1%, etc.) into ALP vial.
4. Replace the cap and shake vigorously for 60 seconds to mix contents until completely dissolved (ideally using a vortex).
5. Sample may be used immediately. Mix vigorously before each use.

a. Store the vial at refrigerated temperatures (2 – 8 °C) for up to 7 days.

III. Test Procedure for Control Testing:

For this procedure, run 3 replicates from each Positive Control and Negative Control sample.

1. Allow six (6) ZymoSnap ALP test devices to equilibrate to room temperature (20 – 25 °C) for 10 minutes.
2. Negative Control Test:
   a. Lift the Snap-Valve bulb out of the tube.
   b. Pipette 75 µL of the Negative Control into the ZymoSnap ALP tube.
   c. Follow the ZymoSnap ALP product instructions for activation and measurement in EnSURE Touch. (Note: Set a name for the sample type/product type on the EnSURE Touch/EnSURE before measurement.)
   d. EnSURE Touch/EnSURE records these results and syncs to the cloud (SureTrend® Cloud) if enabled. Repeat for a total of 3 replicates. The average of 3 results is the background level and is also referred to as the Negative Factor.
   e. If the average value for the Negative Control is not below 20 RLUs, preparation of the Negative Control must be repeated.
3. Positive Control Test:
   a. Lift the Snap-Valve bulb out of the tube.
   b. Pipette 75 µL of 350 mU/L ALP Positive Control into ZymoSnap ALP test device tube.
   c. Follow ZymoSnap ALP product instructions for activation and measurement in EnSURE Touch.
   d. Record RLU results. Repeat for a total of 3 replicates. The average of 3 results is a typical RLU result for the regulatory ALP limit and is the Fail RLU level for this milk type.

Interpretation of Results

The average RLU value from the Positive Control tests is a Fail RLU limit for ZymoSnap ALP test devices (i.e., equal to the regulatory limit of 350 mU/L). The average RLU result obtained from 3 replicate Positive Control tests should fall within the Min-Max RLU values in the below table. If the average RLU result obtained is outside of the range, repeat 3 additional tests from the same positive control vial (as stated in Section III 3).

Milk Fat %	Min RLU	Max RLU
4%	222	249
2%	315	385
0.1%	548	784

Input the Positive Control RLU value into the luminometer for each specific milk type. This same value can be programmed into the luminometer as both Pass and Fail RLUs to give a single Pass/Fail action level.

Storage & Shelf Life

1. Store Positive Control vials at 2 – 8 °C.
2. For Positive Controls, check the expiration date on the box label. Do not use it after the expiration date.
3. The Negative Control should be used within 48 hours when stored at 2 – 8 °C.
4. Reconstituted Positive Controls must be used within 7 days when stored at 2 – 8 °C.

Caution & User Responsibility

The components of the ZymoSnap ALP Positive Control Kit do not pose any health risk when used correctly. Used devices should be disposed of in compliance with Good Laboratory Practice and local Health and Safety Regulations.

1. Ensure proper incubation time and temperature for Negative Control preparation.
2. Ensure Positive Controls are reconstituted with the Negative Control made with milk type to be tested (whole, 2%, 1% or non-fat).

3. Ensure proper storage temperature of Negative and reconstituted Positive Controls.
4. If Negative Controls are stored frozen, aliquot into single-use volumes before freezing. Do not use after one freeze/thaw cycle. The use of freshly made Negative Control is recommended.
5. High RLU results may be produced by reactivated phosphatase and microbial phosphatase. Reactivated phosphatase is a natural phenomenon of ALP when not cooled quickly after pasteurization and/or pasteurized product is stored at temperatures above 8 °C. It is important to maintain samples at chilled temperatures when not being tested. Microbial phosphatase can survive pasteurization when raw milk is contaminated and/or abused prior to pasteurization.

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Contact Information

If more information is required, please visit our help page, email us at techsupport@hygiena.com or contact us by phone at 805.388.8007.