

Positive Control Kit for ZymoSnap™ ALP (Alkaline Phosphatase)

Catalog # ZS-ALP-PC (for use with Hygiena EnSURE Luminometer)



Description/Intended Use:

Positive Control Kit for ZymoSnap ALP (Alkaline Phosphatase) devices consists of 5 vials of one concentration of ALP (350mU/L). This is the globally recognized regulatory maximum for ALP levels in pasteurized milk products. Each vial of Positive Control is reconstituted with a Negative Control, from milk being tested by the user. This will provide a background reading that can be used as a baseline. Positive Control confirms 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 350mU/L regulatory level. A Positive and Negative Control should be run initially to establish a 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 set at $\geq 95^{\circ}\text{C}$ (for preparation of Negative Control)
2. Incubator set at $55^{\circ}\pm 1^{\circ}\text{C}$ (for test procedure)
3. Ice bath for cooling
4. ZymoSnap ALP test devices (Part No. ZS-ALP-100)
5. EnSURE Luminometer (Part No. ENSURE)

I. Preparation of Negative Control:

1. Heat milk sample by either of the following methods:
 - a. Heat 5 mL of milk sample in a 0.5" x 4.0" test tube in an incubator or water bath set at $\geq 95^{\circ}\text{C}$ for 10 minutes.
 - b. Heat any volume of milk to $\geq 95^{\circ}\text{C}$ and maintain for 1 minute while stirring.
2. Cool rapidly on ice, cover, and store at $2^{\circ} - 8^{\circ}\text{C}$ for up to 48 hours. Rapid cooling and cold storage is critical to avoid reactivating ALP.
 - a. Negative Control can be stored frozen at -20°C for up to 3 weeks.
 - b. Negative Control should be used to reconstitute Positive Control vials for testing corresponding milk type (4%, 2%, 1% etc).

II. Preparation of Positive Control:

1. To avoid confusion, label each ALP vial with milk type to be tested.
2. Carefully remove 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 cap and shake vigorously for 10 seconds to mix contents until completely dissolved.
5. Sample may be used immediately. Mix vigorously before each use.
 - a. Store vial at refrigerated temperatures ($2^{\circ} - 8^{\circ}\text{C}$) for up to 7 days.

III. Test Procedure for Control Testing:

1. For this procedure, you will run 3 replicates from each Positive Control and Negative Control sample. Allow 6 ZymoSnap ALP test devices to equilibrate to room temperature ($20^{\circ} - 25^{\circ}\text{C}$) for 10 minutes.
2. Negative Control Test:
 - a. Lift Snap-Valve bulb out of tube.
 - b. Pipette 75 μL of Negative Control into ZymoSnap ALP tube.
 - c. Follow ZymoSnap ALP kit insert instructions for activation and measurement.
 - d. Record RLU results. Repeat for a total of 3 replicates. Average of 3 results is background RLU level and also referred to as the Negative Factor.
3. Positive Control Test:
 - a. Lift Snap-Valve bulb out of tube.
 - b. Pipette 75 μL of 350mU/L ALP Positive Control into ZymoSnap ALP test device tube.
 - c. Follow ZymoSnap ALP Kit Insert instructions for activation and measurement.
 - d. Record RLU results. Repeat for a total of 3 replicates. Average of 3 results is typical RLU result for the regulatory ALP limit and is the Fail RLU level for this milk type.

Interpretation of Results:

Average RLU value from Positive Control tests is Pass/Fail RLU limit for ZymoSnap ALP test devices.

Input Positive Control RLU value as both Pass and Fail RLU limit into luminometer for each specific milk type. For example, if Positive Control RLU value is 150 RLU for 1% milk, then luminometer should be programmed as illustrated in Example 1.

Example 1: Pass/Fail RLU for ZymoSnap ALP Testing

Location	Lower (Pass)	Upper (Fail)
ALP 1% Milk	150	150

Data in Example 1 is for example only. In this example, results less than 150 RLU will be considered Pass; results 150 RLU or greater will be considered Fail.

Storage & Shelf Life:

1. Store Positive Control vials at $2^{\circ} - 8^{\circ}\text{C}$.
2. Positive Controls have a shelf life of 12 months. Check expiration date on box label.
3. Negative Control should be used within 48 hours when stored at $2^{\circ} - 8^{\circ}\text{C}$.
4. Reconstituted Positive Controls must be used within 7 days when stored at $2^{\circ} - 8^{\circ}\text{C}$.

Caution & User Responsibility:

Components of 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. 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.

Hygiena Liability:

Hygiena will not be liable to user or others for any loss or damage whether direct or indirect, incidental or consequential, from use of this device. If this product is proven to be defective, Hygiena's sole obligation will be to replace product, or at its discretion, refund the purchase price. Promptly notify Hygiena within 5 days of discovery of any suspected defect and return product to Hygiena.

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