



Instructions for Use

ALKALINE PHOSPHATASE KIT

Cat. no. Z132	Alkaline Phosphatase Kit	125 tests/kit
	Each kit contains:	
	Z132A - Test Reagent, 0.5oz. Polyethylene, Dropper Bottle 7.0ml	1 bottle
	Z132B - Standard Reagent, 0.5oz. Polyethylene, Dropper Bottle, 7.0ml	1 bottle
	Z132C - Color Developer, 0.5oz. Polyethylene, Dropper Bottle, 14.0ml	1 bottle

INTENDED USE

Hardy Diagnostics Alkaline Phosphatase Kit is recommended for confirmation of successful pasteurization in milk, butter, cream and cheese, using the Rutgers method.⁽¹⁾

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Alkaline phosphatase is an enzyme naturally produced by mammary cells and present in cow's milk. The concentration of alkaline phosphatase can vary based on feed, season, breed of cows, stage of lactation, and milk yield. Alkaline phosphatase is inactivated at a higher temperature than is required to kill non-spore-forming bacterial pathogens. Decreased activity of alkaline phosphatase indicates that the milk has been exposed to enough heat to kill non-spore-forming bacterial pathogens. Alkaline phosphatase activity assays can therefore be used to determine if the milk was pasteurized properly and if the pasteurized milk has been contaminated with raw milk. Alkaline phosphatase is detected by its ability to cleave phenolphthalein monophosphate releasing phenolphthalein. Phenolphthalein is pink in alkaline conditions.

There are several methods that have been developed for the detection of alkaline phosphatase. Hardy Diagnostics Alkaline Phosphatase Kit is based on the Rutgers method, which is a stable and sensitive method that provides an easy to interpret color read out within 30 minutes.⁽¹⁾

This method is also often used to detect the contamination of pasteurized milk by unpasteurized (raw) milk, as well as, pasteurization confirmation.

FORMULA

Test Reagent (Z132A)	
2-Amino-2-Methyl-1-Propanol	73.2gm

Phenolphthalein Monophosphate	3.9gm
Hydrochloric Acid	21.9ml

Final pH 10.0 +/- 0.3 at 25°C.

Standard Reagent (Z132B)	
2-Amino-2-Methyl-1-Propanol	73.5gm
Tartrazine	40.0mg
Phenolphthalein	10.0mg
Hydrochloric Acid	21.9ml

Final pH 10.0 +/- 0.3 at 25°C.

Color Developer (Z132C)	
Sodium Hydroxide	10.0gm
Deionized Water	100.0ml

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. Product should not be used if there are any signs of precipitate, contamination, discoloration, or if the expiration date has passed.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.

Refer to the document "[Storage](#)" for more information.

PRECAUTIONS

All samples of milk and milk products must be held at less than 4.4°C. from the time of collection until sampling and analysis; this will prevent microbial growth, which can cause false-positives, due to the production of microbial alkaline phosphatase.

Analysis must be started within 36 hours of collection.

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

For additional information regarding specific precautions for the prevention of the transmission of all infectious

agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

Refer to the document [SDS Search](#) instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

A positive control and a negative control should be run with each test. To prepare the positive control add 0.2ml of fresh raw milk to 100ml of milk that has been heated at 95°C. for 1 minute. To prepare the negative control heat 5ml of milk or 5gm of milk product to 95°C. for 1 minute. For testing other dairy products, such as sour cream, ice cream, cheese, butter, etc., see listed references.⁽³⁾

1. Pipette 1ml of sample into each of the two test tubes. Label one tube "Test" and the other tube "Standard".
2. Place the tubes in a waterbath or incubator and warm to 37°C.
3. Add 1 drop (0.04ml) of Test Reagent to the tube marked "Test", and 1 drop (0.04ml) of Standard Reagent to the tube marked "Standard". Mix thoroughly by tapping the bottom of the tube.
4. Incubate the tubes at 37°C. for 30 minutes.
5. Add 1 drop (0.04ml) of Color Developer to each tube and mix thoroughly.
6. Visually compare the color of the "Test" to the color of the "Standard".

INTERPRETATION OF RESULTS

A positive result is determined by a color in the "Test" tube that is pink or more pink than the color of the "Standard" control tube. This indicates that there is still alkaline phosphatase present in the milk and it has either not been pasteurized or has been contaminated by unpasteurized milk.

If the "Test" solution is white or less pink than the "Standard" control it is interpreted as a negative test for the presence of alkaline phosphatase. This indicates that the alkaline phosphatase has been inactivated by pasteurization.

The "Standard" approximates pasteurized milk that contains 0.1% raw milk. The positive control consists of pasteurized milk that contains 0.2% of raw milk.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

Note: The alkaline phosphatase content of mixed-herd milk may vary with the breed of cows and the season.

This test is intended to be used as an indicator that milk has been properly pasteurized. It does not guarantee that all organisms have been destroyed.

All samples must be placed in clean containers, refrigerated, and tested within 36 hours in order to be certain that no development of microbial phosphatase has occurred. Exposure to temperatures above 4°C. could lead to false-positives due to the presence of microbial contamination that can produce alkaline phosphatase.

Alkaline phosphatase methodology is applicable to cheese. However, one must be certain that no mold is evident. In

addition, it is highly recommended that cheese be sampled before the addition of condiments, such as peppers or spices, as these materials may also be responsible for false-positive tests.⁽¹⁾

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard laboratory supplies and equipment such as pipettes, test tubes, waterbaths, and incubators, etc., are not provided.

QUALITY CONTROL

This product is tested for sterility and acceptable pH. Refer to the above section, entitled "Formula", for applicable pH range.

Check for signs of contamination and deterioration.

PHYSICAL APPEARANCE

- Test Reagent (Z132A) should appear clear, and yellow in color.
- Standard Reagent (Z132B) should appear clear, and orange in color.
- Color Developer (Z132C) should appear clear and colorless.

REFERENCES

1. Murthy, G.K., et al. 1992. *Standard Methods For The Examination of Dairy Products*, 16th ed. R.T. Marshall, PhD., editor, Chapter 14, p. 425-426. American Public Health Association, Washington, D.C.
2. Wong, K.A. 2005. *Phosphatase Test on Milk Samples to Determine Proper Pasteurization*. California Polytechnical State University, San Luis Obispo, C.A.
3. Murthy, G.K., et al. 1992. *Standard Methods For The Examination of Dairy Products*, 16th ed. R.T. Marshall, PhD., editor, Chapter 14, p. 421-422. American Public Health Association, Washington, D.C.

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1430 West McCoy Lane, Santa Maria, CA 93455, USA

Phone: (805) 346-2766 ext. 5658

Fax: (805) 346-2760

Website: www.HardyDiagnostics.com

Email: TechService@HardyDiagnostics.com

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