WEBER SCIENTIFIC

PHOS-KIT INSTRUCTIONS

For Testing Pasteurization Efficiency by the Scharer Rapid Phosphatase Method

The phosphatase test is performed to determine whether pasteurization was done properly and whether raw milk was added to pasteurized milk.

Alkaline phosphatase is a metabolic product of mammary cell metabolism and a natural constituent of milk. It is heat labile and has an optimum pH of 9.65 to 10.0.

The thermal resistance of phosphatase is greater than that of nonspore-forming pathogenic microorganisms. Therefore, minimum heat treatments applied commercially to inactivate phosphatase will kill all nonspore-forming pathogenic microorganisms. Milk products showing a negative test for phosphatase are considered properly pasteurized and safe.

PRINCIPLE OF METHODS

Tests for phosphatase are based on the principle that the enzyme will hydrolyze monophosphate esters at the proper temperature and pH liberating compounds that can be detected by color development. The amount of color developed is proportional to the enzyme concentration.

In the case of the Scharer Rapid Phosphatase test, any phosphatase present reacts with the substrate disodium phenyl phosphate to liberate phenol. The phenol then reacts with 2,6-dichloroquinone chloroimide (CQC) and copper catalyst to produce indophenol blue.

INSTRUCTIONS

GLASSWARE

All glassware should be scrupulously clean and protected from contamination during storage. Use only detergents that do not contain phenolic compounds. The glassware provided with this kit includes closures with liners that are phenol free.

All glassware should be thoroughly washed and rinsed before being replaced in the PHOS-KIT to prevent cross-contamination from a previous positive test.

CONTROLS

For each series of samples tested, a set of controls must be tested to ensure that the results are true.

Positive Control -- To ensure that the reagents are functioning properly. Add 0.5 ml raw milk to 250 ml boiled milk. A phosphatase test on this sample should have the same color intensity as the #2 Unit color standard. If raw milk is not available, add one drop of a 0.005% phenol solution to 0.5 ml boiled milk and perform the phosphatase test.

Negative Control – To ensure that reagents are free of contaminants.

Place 5 ml of liquid milk sample or 5 g of solid milk sample in a screw-cap test tube. Bring sample to 95°C in a boiling water bath, heat for one minute, then cool rapidly to room

temperature. A phosphatase test run on this sample must give a negative result.

Interfering Substance Control – To test for coloring materials or the effects of fat. Dissolve one buffer control tablet (item #4057-00) in 50 ml distilled water and run the phosphatase test on this solution. Foreign substances or additives may cause a false positive. Development of blue color indicates extent of color due to interfering substances and not to presence of phosphatase. This color should be taken into consideration when interpreting results.

REAGENTS

Buffered Substrate - Place one Buffer Substrate Tablet (item #4058-00) in a glass bottle graduated at 50 ml (provided with kit). Add distilled water to mark, allow tablet to soften then crush with a crystal stirring rod. Shake until completely dissolved. This makes enough solution for ten tests.

Buffer Control - Place one Buffer Control Tablet (item #4057-00) in a glass bottle graduated at 50 ml (provided with kit). Add distilled water to mark, allow tablet to soften then crush with a crystal stirring rod. Shake until completely dissolved. This makes enough solution for ten tests.

CQC - Place one CQC Tablet (item #4059-00) in a test tube. Add 5 ml *methyl alcohol*. Allow tablet to soften then crush with a crystal stirring rod. Shake vigorously until dissolved, transfer to a clean amber glass dropper bottle (provided with kit) and store in the refrigerator. Disregard any undissolved copper catalyst. This solution makes enough for 25 - 30 tests.

Discard solution when it begins to turn brown. Rinse bottle and dropper with methyl alcohol before re-using. NOTE: Ethyl alcohol cannot be used because it does not dissolve the copper catalyst.

METHODS

All samples must be held at 0° to 4.4°C or less from the time of collection to the time of analysis.

Test tubes are calibrated for the TOP of the meniscus to meet the mark. Calibration marks indicate 5.0, 5.5 and 8.5 ml.

1. Fill the test tube to the 5.0 ml mark with Buffer Substrate solution adjusting the level with a glass dropper pipet.

2. Add 0.5 ml of sample using a fresh glass dropper pipet; mix by inverting the tube several times. NOTE: use a fresh glass dropper pipet for each sample.

3. Immediately place the tubes in a water bath at 40° ± 1°C. Allow tubes to warm up to 40°, then heat for another 15 minutes. Alternately, incubate for 20 minutes at 40° ± 1°C.

4. Remove the tubes from the water bath and add 6 drops of the CQC solution. Mix the samples by inverting several times and immediately re-incubate the tubes for exactly 5 minutes.

5. Remove the tubes from the water bath and cool them in an ice water bath to below 10°C. Add 3.0 ml cold neutralized N-butyl alcohol.

6. Replace the cap and extract the indophenol by gently inverting the tubes through a half circle: Take about 1 second to invert the tubes, pause about 1 second, take another 1 second to return the tubes upright, pause 1 second, and then repeat three more times. Lay the tubes on their side on a flat surface for 2 minutes to permit the separation of the butyl alcohol and then repeat the mixing and separating steps.

7. If the butyl alcohol has been emulsified so that no clear butyl alcohol layer remains, cool the tubes for 5 minutes in an ice water bath and then centrifuge them for 5 minutes at 1000 x g. Otherwise, repeat the test.

8. Read the results by standing the tubes upright and comparing the color of the samples with the three color standards.

INTERPRETATION

For best results, view the tubes against the white plastic filter screen held in front of direct light source (daylight or fluorescent fixture). Do not attempt to match the sample results to the color standards; rather, determine whether the sample contains more or less blue color than the standards.

Milk or cream that has been properly pasteurized will yield no color. A sample should be considered improperly pasteurized if it yields a color equal to or greater than the #1 unit color standard.

FALSE POSITIVES

Several conditions may cause a false positive result:

- Inadequately cleaned or rinsed glassware.
- Closures that contain phenolic resins. The bottles and test tubes included in this kit are phenol free.
- Old or contaminated test reagents. Store the solutions tightly closed in the refrigerator.
- Improperly stored reagent tablets. The tablets come packed with a desiccant. Store the bottles tightly closed and protected from dampness, heat and sunlight. Failure to store them properly could lead to deterioration.

FALSE NEGATIVES

- High Temperature. If samples are incubated at too high a temperature, the effect of the test may be lost. Boiling destroys the enzymes in the same manner as proper pasteurization.
- Color Development Time. This is an enzymatic reaction; the amount of phenol liberated by phosphatase is time and temperature dependent.

SAMPLE PREPARATION OF OTHER MILK PRODUCTS

Cream - Cream samples are treated the same as milk.

Cultured Dairy Products - Homogenize cultured buttermilk, sour cream and yogurt in a blender for a few seconds, avoiding whipping. Use double strength buffered substrate and perform the test as described for milk.

To make double strength buffered substrate, dissolve the buffered substrate tablet in 25 ml

water.

Ice Cream - Melt a portion of the sample and let it stand for an hour to release any trapped air. Remove any fruit or nuts before testing. Treat each flavor individually. Perform the test as described for milk using the single strength buffered substrate.

Butter - Freeze portions of butter to be tested at -15°C ± 1°C. Weigh 0.5 g samples directly into test tubes. Heat the negative control at 95°C for 1 minute in a boiling water bath, then cool in an ice water bath.

Place the negative control and the test samples in a 40°C water bath until the samples melt before adding buffered substrate. Test as with milk using single strength buffered substrate.

Cheese - Measure the pH of the cheese sample to be tested. Freeze portions of cheese to be tested at -15°C ± 1°C. Weigh 5.0 g samples directly into test tubes. Add 2.0 ml of water to each sample of cheese with a pH above 7.0; add 2.0 ml carbonate buffer* to each sample of cheese with a pH below 7.0. Macerate the sample with a crystal stirring rod.

Heat the negative control at 95°C for 1 minute in a boiling water bath, then cool in an ice water bath.

Add 18 ml of 8.3% neutralized n-butyl alcohol to the negative control and the samples. Mix on a vortex mixer then let stand for 5 minutes. Filter samples through Whatman #4 filter paper into clean test tubes placed in a water bath.

Add 0.5 ml portions of filtrate into test tubes and add 0.1 ml of magnesium acetate solution**. Use double strength buffered substrate solution. Proceed as with milk.

*To make carbonate buffer, add 46.89 g sodium carbonate and 37.17 g sodium bicarbonate to enough water to make 1 liter of solution. Stored in a glass-stoppered bottle, this solution in stable indefinitely.

**To make magnesium acetate solution, dissolve 8.82 g magnesium acetate in 50 ml water. Quantitatively transfer the solution to a 100 ml volumetric flask and dilute to 100 ml with water.

THE WEBER SCIENTIFIC PHOS-KIT includes the following:

1 removable 9-tube rack (item #4054-00)
9 graduated test tubes with phenol-free screw caps (item #4052-01)
1 set of 3 permanent color standards (item #4054-05)
1 plastic filter sheet (item #4054-10)
4 crystal stir rods (item #4054-25)
12 glass dropping pipets with rubber bulbs (item #4054-15)
1 amber glass dropping bottle (item #4054-30)
1 amber glass bottle for 1 oz. butyl alcohol (item #4054-35)
1 50 ml graduated bottle (item #4054-40)
50 Buffer Substrate Tablets (item #4058-00)
50 CQC Reagent Tablets (item #4059-00)
50 Buffer Control Tablets (item number 4057-00)