Microbial and Reactivated ALP

Detection of Microbial Phosphatase

Pipette 1 - 5ml of sample into a labelled test tube, then replace cap. Place the container in the water bath set at 63°C (66°C if the fat content exceeds 10%; this will be applicable to all cream samples). Ensure that at least two-thirds of the container is below the level of the water, or the water level is at least 4cm above the sample level

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Heat for 30 minutes, gently mixing the sample every 10 minutes. Remove the container from the water bath and cool for at least 5 minutes in cold water

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Retest the sample for phosphatase activity as described above. If the phosphatase level of this heated sample still exceeds 100mU/l the reading is due to the presence of microbial phosphatase and the original sample was properly pasteurised

Differential Test for Reactivation

Reagent - Magnesium Acetate (40.1mg of Mg++/ml): Dissolve 35.4g of $Mg(C_2H_3O_2)_2.4H_2O$ in about 50ml of water. Warm to dissolve completely, then bring to 100ml with additional DI water. This solution is stable for 6 months at 2-9°C

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Place 10ml of the sample in a suitable glass container and heat in a boiling water bath for 1 minute after the test product temperature reaches 95°C. Cool rapidly for 5 minutes

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Place 5.0ml of unheated sample in each of two test tubes. Label one tube “blank” and add 0.1ml of deionised water. Label the second tube “test” and add 0.1ml of magnesium acetate solution. Cap both tubes and mix well using the vortex mixer. Incubate at 34°C for 1 hour. Remove the test tubes and cool rapidly for 5 minutes

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Perform a phosphatase test on the “blank” sample as described above. Add 1.0ml of the “test” sample to 5.0ml of heated, cooled test product (1 + 5 dilution). Perform a phosphatase test on this “diluted test” sample

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If the phosphatase activity of the “diluted test” sample (1 + 5 dilution) containing magnesium ions has equal or greater activity than the undiluted sample containing no magnesium ions (the “blank” sample), the original product is considered negative for residual alkaline phosphatase activity, indicating that the phosphatase level originally measured is of reactivated origin

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If the “diluted test” sample contains less phosphatase activity than the undiluted sample, it is considered positive for residual mammalian phosphatase (provided that the initial alkaline phosphatase test was positive)

Note: A false positive test for residual phosphatase may be obtained if a reactivatable sample is stood at elevated temperature (21 - 24°C) for 2 hours.