PHOSPHATASE TEST – CHARM® FAST ALKALINE PHOSPHATASE TEST USING CHARM NOVALUM®
IMS #29

[Unless otherwise stated all tolerances are ±5%]

SAMPLES

1. Laboratory Requirements (see Cultural Procedures [CP], items 33 & 34) _____
[See current version of M-a-98 to determine if this test method has been approved for use on the specific dairy product being tested]

a. Product Groups/Descriptions _____

1. Fluid white milks - including skim through whole fat milk _____

2. Unflavored liquid dairy products – including half and half, cream, light cream, whipping cream (products that can be accurately pipetted) _____

3. Flavored liquid dairy products (Liquid products that can be accurately pipetted, containing flavor additives and/or thickening agents including flavored milk, and etc.) _____

APPARATUS

2. CP, items 1-32 (as necessary) _____

a. Unless otherwise stated, “shake vigorously” refers to standard microbiological mixing, i.e., 25 times in a 1 foot arc in 7 sec or vortex for 10 sec at maximum setting (subsamples/controls in an appropriate container for vortexing) _____

3. Pipettors and Pipets _____

a. Fixed volume or electronic, 100 µL _____

b. Calibration checked as specified in CP item 6.e; maintain records _____

c. Disposable, 10 mL (ASTM) pipet with 0.1 mL graduations _____

4. Microtube Adapter for NovaLUM _____

5. NovaLUM Analyzer _____

a. Operating instructions available _____

1. Channels configured for Fast Alkaline Phosphatase (FAP) assay for appropriate definitions _____

a. FAP MILK – 45 sec time _____

b. FAP CREAM – 90 sec time _____

c. FAP CHOC – 90 sec time _____

2. Thermoprobe connected with NovaLUM positioned upright in Stand _____

a. Probe measuring ambient room temperature, DO NOT IMMERSE IN WATER (Ambient room temperature must be between 18-24ºC to run the test) _____

3. Microtube adapter for Luminometer/Luminator/NovaLUM _____

6. Water Bath, Circulating, 34±1ºC and 63±1ºC (or 66±1ºC if fat > 10%), or 13 x 100 Test Tube Dry Well Heater Blocks Acceptable (Confirmation Procedure) _____

7. Centrifuge - Charm II Heraeus® (3,400 RPM), Minifuge, or Equivalent (1,200-2,000 g) _____

8. Handling and Storage _____

a. Kit contains Reagent FAP Vials and Calibrator Tablets _____

Kit: Lot #: _____ Exp Date: ___/___/___ _____

Calibrator Lot #: _____ Exp Date: ___/___/___ _____

b. Reagents stored at 0.0-4.5ºC until expiration date _____

1. FAP vials may be stored at room temperature. If stored at room temperature, laboratory expiration date is 3 weeks from first date of room temperature storage. FAP vials must be at 18-24ºC at time of use _____

c. Label bottles with open dates _____

CONTROLS

9. Negative Calibrator/Control _____

a. Product group. Prepare at least 20 mL of negative sample for use as a negative calibrator/control and to rehydrate 350mU/L positive calibrator/control _____

1. Fluid white milk - heat a sample of product (highest fat content) to 95±1ºC for 1 min with stirring _____

2. All flavored liquid dairy products can be tested on the FAP CHOC channel by heating a chocolate sample (highest fat content) to 95±1ºC for 1 min with stirring _____

a. Cool rapidly in an ice bath and hold at 0.0-4.5ºC _____

b. Centrifuge for 3 min and decant supernatant _____

3. All unflavored liquid dairy products can be tested on the FAP CREAM channel by heating pasteurized light cream to 95±1ºC for 1 min with stirring _____

4. Note: if product precipitates during negative sample preparation, e.g. sheep milk, heating sample to 63ºC for 45 min is acceptable. If using 13 x 100 test tube dry well heater block at 95ºC, it takes 10 min to heat product to 95ºC; once at temperature, time for 1 min (Use TC) _____

b. Cool rapidly in an ice bath and hold at 0.0-4.5ºC _____

c. Store at 0.0-4.5ºC, the Negative Control/Sample may be used for up to 48 hours _____

d. Or, aliquot 1 mL quantities into small tubes (see 5.a.2.b for product definitions), seal and freeze at –15ºC or colder in a non-frost-free freezer or in an insulated foam container in a frost-free freezer, use within 2 months _____

Lab Prep. Date:_____ Lab Exp. Date:_____ _____

10. Positive 350 mU/L Calibrator/Control _____

a. Prepare Positive Calibrator/Control _____

1. Rehydrate a calibrator tablet with 100 µL water, mix to disperse tablet, wait 1 min and mix again _____

2. Add 2.5 mL of Negative Calibrator/Control to dissolve calibrator tablet _____

3. Shake vigorously and let settle 10 min at 0.0-4.5ºC for re-suspension _____

4. Shake vigorously again and use for test _____

b. Positive calibrator/control held at 0.0-4.5ºC may be used for 48 hours _____

CALIBRATION

11. With Each New Kit Lot # Calibrate Analyzer and Replace Microtube Adapter _____

a. Prepare Negative Calibrator/Control and Positive Calibrator/Control, items 9 and 10 _____

b. Select appropriate channel for calibration and follow prompts.
Note: Previously calibrated channels will list a selection menu, select 'calibrate'; follow prompts _____

1. Test a negative calibrator/control, item 13.c _____

2. Test a positive calibrator/control, item 13.c _____

3. Instrument will make internal adjustments _____

4. Test another negative calibrator/control, item 13.c _____

5. Test another positive calibrator/control, item 13.c _____

6. If performance of negative (<15) and positive is in range (320-400), instrument will prompt calibration successful. If performance out of range, instrument will recalculate settings and prompt to perform another positive and negative calibrator/control _____

7. Repeat steps 4-6. If out of range NovaLUM will prompt a re-calibration, step 1 _____

DAY OF USE PERFORMANCE CHECKS

12. Each Day of Use, Test a Negative Control/Sample (item 9) and Positive Control (item 10), For at Least One Product _____

a. Verify FAP vial stored at room temperature. Select NovaLUM 'programmed plans', select appropriate FAP channel and select menu 3 'Control Check'. Follow Prompts _____

1. Test positive calibrator/control, item 13.c. Positive Control valid, 247-453 mU/L _____

2. Test negative calibrator/control, item 13.c. Negative Control valid or less than or equal to 15 mU/L _____

TEST PROCEDURE

13. Procedure _____
[Samples kept at 0.0-4.5ºC throughout testing]

a. Prepare sample _____

1. Mix retail milk samples by inverting containers top to bottom, then bottom to top (a complete half circle or 180 degrees) without pausing, 25 times; use within 3 min _____

2. Mix negative control or subsamples of retail containers by shaking 25 times in 7 sec with a 1 ft movement or vortex at least 10 sec at maximum setting; use within 3 min. (sample(s)/control(s) must be in appropriate container to allow the use of vortexing) _____

3. For flavored dairy products (not including controls, items 9 & 10) _____

   a. Add 1 mL of sample into an appropriate tube or vial (NOT FAP vial) _____

   b. Centrifuge for 3 min _____

   c. Use liquid extract in item 13.d _____

b. Verify FAP vial stored at room temperature _____

   1. Pierce foil top with clean pipet tip _____

c. Dispense 100 µL of the prepared sample (item 13.a) or mixed controls (items 9 & 10) into the FAP vial liquid and then immediately press enter on NovaLUM _____

   1. Follow prompt and vortex FAP vial with sample for 5 sec at maximum setting _____

   2. Follow prompt and attach microtube adapter to threaded side of vial. Then fully insert vial into NovaLUM chamber. This step must be completed while screen is flashing (30 sec) _____

d. At the end of pre-programmed time, the screen will stop flashing and count the sample. The mU/L phosphatase level will be displayed on screen. Press OK to print and prepare for next sample _____

e. Samples with ≥ 350 mU/L of ALP activity are suspect positive and must be confirmed (item 14) _____

CONFIRMATION

14. Positive Confirmation _____

a. Prepare lab pasteurized negative control and positive control made of the same dairy product _____

b. Test controls to verify they are in range. If out of range, recalibrate channel and test controls to verify calibration _____

c. Retest suspect positive sample _____

d. Samples with ≥ 350 mU/L of ALP activity are suspect positive and must be tested for microbial, and reactivated phosphatase (items 15 & 16) _____

15. Microbial Phosphatase/Heat Stable Phosphatase _____

a. Heat 1.0 mL of suspect sample at 63±1ºC for 30 min, stirring or mixing every 10 min (Use TC) _____

   1. If fat content is >10%, heat at 66±1ºC for 30 min _____

b. Cool sample rapidly to 0.0-4.5ºC in an ice bath _____

c. Test positive and negative controls (item 14.a) following item 13 _____

d. Test heated sample and unheated sample (original sample) following item 13 _____

e. Interpretation _____

1. Controls test as specified in item 12 _____

2. If heated and unheated samples have equal activity (-30%,mU/L or RLU) the sample is regarded Not Found for residual phosphatase, the activity originally measured is microbial _____

3. If the heated sample is more than 30% below unheated sample (mU/L or RLU), the sample contains milk phosphatase activity, either residual or reactivated _____

16. Reactivated Phosphatase _____

a. Magnesium acetate solution commercially available _____

b. Or, prepared in laboratory _____

1. Dissolve 35.4 g of Mg acetate tetra-hydrate, Mg $(C_2H_3O_2)_2 \cdot 4H_20$ in 25 mL deionized (DI) water, warming slightly to aid dissolution _____

2. Pour solution into 100 mL volumetric flask, rinse original container several times and add rinses to flask _____

3. After cooling to room temperature, make up to 100 mL (stable for 1 year at 0.0-4.5ºC) _____

c. Procedure _____

1. Add a 5.0 mL aliquot of sample (unheated, original sample not prepared as in 13.a) to each test tube _____

2. Add 0.1 mL DI water to the sample labeled "Blank", and 0.1 mL Mg acetate solution to the sample labeled "Test" _____

3. Cap tubes, mix and heat both aliquots for 1 hour at 34±1ºC (Use TC) _____

4. Remove samples from water bath and cool rapidly to 0.0-4.5ºC in an ice bath _____

5. Dilute 1 mL of sample containing Mg acetate (Test) with 5 mL (1:6 dilution) of negative control product (item 14.a) and mix, label tube as "Diluted Test" _____

6. Test undiluted sample containing no Mg acetate (Blank) and diluted sample containing Mg acetate (Diluted Test) for phosphatase activity following item 13 _____

d. Interpretation _____

1. If the diluted aliquot containing Mg acetate (Diluted Test) has equal (±30%) or greater phosphatase activity than the undiluted aliquot containing no Mg acetate (Blank), the sample is regarded as Not Found for residual phosphatase, and the phosphatase originally measured is of **reactivated** origin _____

Diluted w/Mg (Test) ≥ Undiluted (Blank) = Reactivated

2. If the diluted aliquot (Diluted Test) contains less (30% below or less) activity than the undiluted aliquot (Blank) the sample is considered Positive for **residual phosphatase** _____

Diluted w/Mg (Test) < Undiluted (Blank) = Residual

3. A false-positive for residual phosphatase may also be obtained if a reactivatable sample has been allowed to stand at elevated temperatures (20C) for periods of 1 hour or more before testing (SPC < 20,000/mL) _____

RECORDING, INTERPRETATION, AND REPORTING

17. Recording and Interpretation _____

a. Record Values _____

b. Interpret _____

1. If value obtained is <44 mU/L for fluid white milk or <88 mU/L for flavored/unflavored the sample is Not Detected _____

2. If value obtained is ≥350 mU/L or mU/kg the sample is **actionable** _____

18. Report _____

a. **Not Found** for residual phosphatase if: _____

1. <350 mU/L _____

2. ≥350 mU/L but: _____

a. Meets reactivated phosphatase criteria (item 16.d.1) _____

b. Meets microbial phosphatase criteria (item 15.e.2) _____

c. Documentations showing the products was treated in such a way that reactivated phosphatase may be present _____

b. **Positive** for residual phosphatase if: _____

1. ≥350 mU/L or mU/g and: _____

   a. Meets residual phosphatase criteria (item 16.d.2) _____

   b. No microbial phosphatase present (item 15.e.3) _____

   c. No documentation to show the product could have become reactivated _____