

**PHOSPHATASE TEST – CHARM<sup>®</sup> FAST ALKALINE PHOSPHATASE TEST  
USING CHARM NOVALUM<sup>®</sup>  
IMS #29**

[Unless otherwise stated all tolerances are  $\pm 5\%$ ]

**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  $\mu$ L \_\_\_\_\_
- 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 18-24°C to run the test) \_\_\_\_\_
  - 3. Microtube adapter for Luminometer/Luminator/NovaLUM \_\_\_\_\_
- 6. **Water Bath, Circulating, 34±1°C and 63±1°C (or 66±1°C 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 0.0-4.5°C 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 18-24°C 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±1°C 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±1°C for 1 min with stirring \_\_\_\_\_
      - a. Cool rapidly in an ice bath and hold at 0.0-4.5°C \_\_\_\_\_

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  $95\pm 1^{\circ}\text{C}$  for 1 min with stirring \_\_\_\_\_

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

b. Cool rapidly in an ice bath and hold at  $0.0\text{-}4.5^{\circ}\text{C}$  \_\_\_\_\_

c. Store at  $0.0\text{-}4.5^{\circ}\text{C}$ , 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  $-15^{\circ}\text{C}$  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  $\mu\text{L}$  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  $0.0\text{-}4.5^{\circ}\text{C}$  for re-suspension \_\_\_\_\_

4. Shake vigorously again and use for test \_\_\_\_\_

b. Positive calibrator/control held at  $0.0\text{-}4.5^{\circ}\text{C}$  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 0.0-4.5°C 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  $\mu$ L 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  $\geq 350$  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  $\geq 350$  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  $63 \pm 1^\circ\text{C}$  for 30 min, stirring or mixing every 10 min (Use TC) \_\_\_\_\_
  - 1. If fat content is  $>10\%$ , heat at  $66 \pm 1^\circ\text{C}$  for 30 min \_\_\_\_\_

- b. Cool sample rapidly to 0.0-4.5°C 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<sub>2</sub>H<sub>3</sub>O<sub>2</sub>)<sub>2</sub>·4H<sub>2</sub>O 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 0.0-4.5°C) \_\_\_\_\_
- 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±1°C (Use TC) \_\_\_\_\_
  - 4. Remove samples from water bath and cool rapidly to 0.0-4.5°C 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 ( $\pm 30\%$ ) 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)  $\geq$  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)

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### RECORDING, INTERPRETATION, AND REPORTING

#### 17. Recording and Interpretation

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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  $\geq 350$  mU/L or mU/kg the sample is **actionable**

\_\_\_\_\_

\_\_\_\_\_

#### 18. Report

\_\_\_\_\_

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

\_\_\_\_\_

1.  $< 350$  mU/L

\_\_\_\_\_

2.  $\geq 350$  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.  $\geq 350$  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 \_\_\_\_\_