

**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

$$\text{Diluted w/Mg (Test)} \geq \text{Undiluted (Blank)} = \text{Reactivated}$$

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**

$$\text{Diluted w/Mg (Test)} < \text{Undiluted (Blank)} = \text{Residual}$$

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  $\geq 350$  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 \_\_\_\_\_