

**PHOSPHATASE TEST - CHARM® FAST ALKALINE PHOSPHATASE TEST  
USING CHARM NOVALUM® AND NOVALUM II X®  
IMS #28c**

[Unless otherwise stated all tolerances are ±5%]

**SAMPLES**

**1. Laboratory Requirements (see Cultural Procedures [CP], items 34 & 35)** \_\_\_\_\_

[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 1-33 (as necessary)** \_\_\_\_\_

- a. Unless otherwise stated, “shake vigorously” refers to standard microbiological mixing, i.e., 25 times in a 1 foot movement 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/NovaLUM II X** \_\_\_\_\_

**5. NovaLUM/NovaLUM II X 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) or NovaLUM II X \_\_\_\_\_
    - a. Probe measuring ambient room temperature (must be between 18-24°C to run the test) \_\_\_\_\_
  - 3. Microtube adapter for Luminometer/Luminator/NovaLUM/NovaLUM II X \_\_\_\_\_
- 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. **Vortex Mixer** \_\_\_\_\_
- 9. **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 \_\_\_\_\_

**CONTROLS**

- 10. **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, within 24 hours, aliquot 1 mL quantities into small tubes (see 5.a.1 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: \_\_\_\_\_

#### 11. Positive 350 mU/L Calibrator/Control \_\_\_\_\_

a. Prepare Positive Calibrator/Control \_\_\_\_\_

1. Rehydrate a calibrator tablet with 100  $\mu\text{L}$  MS or DI water, mix to disperse tablet, wait 1 min and mix again \_\_\_\_\_

2. Add 2.5 mL of Negative Calibrator/Control (item 10) to dissolve calibrator Tablet \_\_\_\_\_

3. Shake vigorously or vortex and let settle 10 min at  $0.0\text{-}4.5^{\circ}\text{C}$  for re-suspension \_\_\_\_\_

4. Shake vigorously or vortex 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

#### 12. With Each New Kit Lot # Calibrate Analyzer and Replace Microtube Adapter \_\_\_\_\_

a. Prepare Negative Calibrator/Control and Positive Calibrator/Control, items 10 and 11 \_\_\_\_\_

- 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 14.c \_\_\_\_\_
- 2. Test a positive calibrator/control, item 14.c \_\_\_\_\_
- 3. Instrument will make internal adjustments \_\_\_\_\_
- 4. Test another negative calibrator/control, item 14.c \_\_\_\_\_
- 5. Test another positive calibrator/control, item 14.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/NovaLUM II X will prompt a re-calibration, step 1 \_\_\_\_\_

**DAY OF USE PERFORMANCE CHECKS**

**13. Each Day of Use, Test a Negative Control/Sample (item 10) and Positive Control (item 11), For at Least One Product** \_\_\_\_\_

- a. Verify FAP vial stored at room temperature. For NovaLUM, select 'programmed plans', select appropriate FAP channel and select menu 3 'Control Check'. For NovaLUM II X, select FAP from home screen, select appropriate pre-programmed FAP channel, then select 'Control Check' and follow prompts \_\_\_\_\_
- 1. Test positive calibrator/control, item 14.c. Positive Control valid, 247-453 mU/L \_\_\_\_\_
- 2. Test negative calibrator/control, item 14.c. Negative Control valid or less than or equal to 15 mU/L \_\_\_\_\_
- b. Periodic rotation of channels is recommended when multiple channels are used \_\_\_\_\_

**TEST PROCEDURE**

**14. Procedure [Samples kept at 0.0-4.5°C throughout testing]** \_\_\_\_\_

- a. Prepare sample \_\_\_\_\_
- 1. Mix retail milk samples by inverting container 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 10 & 11) \_\_\_\_\_
  - a. Add 1 mL of sample into an appropriate tube or vial (NOT FAP vial) \_\_\_\_\_
  - b. Centrifuge for 3 min \_\_\_\_\_
  - c. Use liquid phase in item 14.c \_\_\_\_\_
- 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 14.a) or mixed controls (items 10 & 11) into the FAP vial liquid and then immediately press enter on NovaLUM or press the 'Run Test' icon on NovaLUM II X \_\_\_\_\_
  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/NovaLum II X 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. For NovaLUM, press OK to print and prepare for next sample. For NovaLUM II X result prints automatically, press the right arrow icon then select 'Run Test' to prepare for the next sample \_\_\_\_\_
- e. Samples with  $\geq 350$  mU/L of ALP activity are suspect positive and must be confirmed (item 15) \_\_\_\_\_

**CONFIRMATION**

**15. 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 16 & 17) \_\_\_\_\_

**16. 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  $0.0-4.5^{\circ}\text{C}$  in an ice bath
- c. Test positive and negative controls (item 15.a) following item 14
- d. Test heated sample and unheated sample (original sample) following Item 14
- e. Interpretation
  - 1. Controls test as specified in item 13
  - 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

**17. Reactivated Phosphatase**

- a. Magnesium acetate solution commercially available
- b. Or, prepared in laboratory
  - 1. Dissolve 35.4 g of Mg acetate tetra-hydrate,  $\text{Mg}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 4\text{H}_2\text{O}$  in 25 mL MS or DI water, warming slightly to aid dissolution
  - 2. Pour solution into 100 mL volumetric flask, rinse original container several times and add rinse to flask
  - 3. After cooling to room temperature, make up to 100 mL (stable for 1 year at  $0.0-4.5^{\circ}\text{C}$ )
- c. Procedure
  - 1. Label two 13 x 100 test tubes for appropriate for volume as "Blank" and "Test"
  - 2. Add a 5.0 mL aliquot of sample (unheated, original sample to each test tube
  - 3. Add 0.1 mL DI or MS water to the sample labeled "Blank", and 0.1 mL Mg acetate solution to the sample labeled "Test"
  - 4. Cap tubes, mix and heat both aliquots for 1 hour at  $34\pm 1^{\circ}\text{C}$  (Use TC)

5. Remove samples from water bath and cool rapidly to 0.0-4.5°C in an ice bath \_\_\_\_\_
6. Dilute 1 mL of sample containing Mg acetate (Test) with 5 mL (1:6 dilution) of negative control product (item 15.a) and mix, label tube as "Diluted Test" \_\_\_\_\_
7. Test undiluted sample containing no Mg acetate (Blank) and diluted sample containing Mg acetate (Diluted Test) for phosphatase activity following item 14 \_\_\_\_\_

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 (20°C) for periods of 1 hour or more before testing (SPC  $<$  20,000/mL) \_\_\_\_\_

### RECORDING, INTERPRETATION, AND REPORTING

#### 18. 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** \_\_\_\_\_

#### 19. Report \_\_\_\_\_

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

1.  $< 350$  mU/L \_\_\_\_\_

2.  $\geq 350$  mU/L but: \_\_\_\_\_

a. Meets reactivated phosphatase criteria (item 17.d.1) \_\_\_\_\_

b. Meets microbial phosphatase criteria (item 16.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 17.d.2) \_\_\_\_\_

b. No microbial phosphatase present (item 16.e.3) \_\_\_\_\_

c. No documentation to show the product could have become Reactivated \_\_\_\_\_