

**PHOSPHATASE TEST - CHARM® FAST ALKALINE PHOSPHATASE TEST
USING CHARM NOVALUM® AND NOVALUM II X®
IMS #28c
FDA/NCIMS Revision 10/19**

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

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°C for 45 min is acceptable. If using 13 x 100 test tube dry well heater block at 95°C , it takes 10 min to heat product to 95°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°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 μL 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 µL 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 ≥ 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 ≥ 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 $0.0\text{--}4.5^\circ\text{C}$ 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 ($\sim 30\%$, 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 $0.0\text{--}4.5^\circ\text{C}$)
- 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 ≥ 350 mU/L or mU/kg, the sample is **actionable** _____

19. Report _____

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

1. <350 mU/L _____

2. ≥ 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. ≥ 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
