PHOSPHATASE TEST - FLUOROPHOS® ALP TEST SYSTEM
IMS #28

[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]

APPARATUS

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

3. Cuvette Heating Block _____

a. Thermostatically controlled at 38±1ºC _____

b. Check temperature and record each day of use _____

4. Pipettors, Fixed Volume or Electronic _____

a. 75 µL pipettor _____

b. 25 µL pipettor, for use with high-turbidity or high fat products (if needed) _____

c. Calibrated as specified in CP item 6.e; maintain records _____

5. Reagent Dispenser _____

a. Fixed volume 2.0 mL; calibrated and checked _____

b. Optionally, use 2.0 mL fixed volume or electronic pipettor to dispense reagent or sterile serological pipette _____

c. Calibrated as specified in CP item 6.e; maintain records _____

6. Cuvettes _____

a. Disposable glass 12 x 75 mm, dirt and scratch free _____

7. Vortex Mixer (optional) _____

8. Fluorometer _____

a. Air fan in the rear unobstructed _____

b. Vents in the bottom base plate are unobstructed _____

c. User's manual available _____

9. Water Baths, 34±1ºC, 63±1ºC, 66±1ºC, Circulating (Confirmation Procedures) _____

REAGENTS

10. Reagents, Handling and Storage _____

a. Test Reagent Set _____

1. Fluorophos substrate and Substrate buffer _____

2. Lot #: _____ Exp. Date: _____ _____

b. Calibrator Set _____

1. Calibrators A, B and C _____

2. Lot #: _____ Exp. Date: _____ _____

c. PhosphaCheck® Pasteurization Controls Set _____

1. Positive and Negative Control _____

2. Lot #: _____ Exp. Date: _____ _____

d. Daily Instrument Control _____

1. Lot #: _____ Exp. Date: _____ _____

e. Store reagents at 0-6ºC _____

REAGENT PREPARATION

11. Working Substrate _____

a. Prepare reagents as per manufacturer instructions (mix by inversion until fully dissolved) _____

b. Date (mixture stable 60 days at 0-6ºC) _____

1. Label bottle with date prepared _____

2. Preparation Date: _____ _____

c. Place clean 2 mL reagent dispenser (item 5) on prepared reagent bottle, or cap if using 2 mL pipettors or sterile serological pipette _____

INSTRUMENT AND REAGENT CHECKS

12. Check Procedures (prior to testing each day of use) _____

a. Zero Check _____

1. A/D value: _____ _____

2. The reading must not exceed 314. If the reading exceeds 314, do not proceed; call for technical assistance _____

3. Maintain records _____

b. Calibrator C/Daily Instrument Control Check _____

1. Use 2.0 mL of Calibrator C (item 10.b) or Daily Instrument Control (item 10.d) that has been warmed to 38±1ºC (approx. 20 min) _____

a. A/D value: _____ _____

b. The A/D value must be 602±15 _____

c. If the value does not fall within the acceptable range, adjust according to manufacturer _____

d. Maintain records _____

c. Reconstituted Substrate/Buffer stability check _____

1. Use 2.0 mL of working substrate (item 11) that has been warmed to 38±1ºC (approx. 20 min) _____

a. A/D value: _____ _____

b. The A/D value must be < 1,200 _____

c. Maintain records _____

d. Reconstituted Substrate/Buffer contamination check _____

1. Use 2.0 mL of working substrate (item 11) that has been warmed to 38±1ºC (approx. 20 min) _____

2. Initiate an ALP sample reading of the working substrate on an unused channel _____

a. ALP value: _____ _____

b. The ALP value must be < 10 mU/L _____

c. If the working substrate value does not fall within the acceptable range, do not use working substrate; re-check to verify, reconstitute a new set of reagents or seek technical assistance before testing samples _____

d. Maintain records _____

CALIBRATION

(Required at Installation and After any Instrument Adjustments)

13. Calibration Procedure _____

a. Perform instrument and reagent checks (item 12) prior to proceeding _____

1. If readings from item 12 are within specification, proceed with calibration _____

2. If readings are not within specification, do not proceed with calibration, make appropriate adjustments or seek technical assistance and re-check _____

3. Record all values (initial and re-checks) in QC record _____

b. Check calibration ratio of Calibrators A, B and C; maintain records _____

1. Add 2 mL of each calibrator to appropriately labeled tubes _____

2. Heat tubes to 38±1ºC for 20 min _____

3. Find an empty channel _____

4. Place a tube of warmed Calibrator A (with no milk added) into the cuvette chamber, close the door and press the "Start" key _____

5. Continue as prompted until all six (6) tubes have been run _____

6. Calibration ratio should be 151±7 (when A/D mode check for Calibrator C/Daily Instrument Control is 602±6) _____

7. If ratio within specification continue, if not make adjustment and re-check calibration ratio _____

c. Check calibration for products by adding 75 µL (or 25 µL) of the well-mixed product to each calibrator one tube at a time just prior to testing (Instrument calibrated for each product type; some products with similar fat content may share same channel) _____

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 subsamples of retail milk containers or controls by shaking 25 times in 7 sec with a 1 ft movement or vortex for 10 sec at maximum setting (subsamples in appropriate containers to allow the use of vortexing); use within 3 min _____

3. Remove test portion(s) avoiding foam and bubbles _____

    a. For positive displacement pipettors with reusable tip(s)

        1. Prior to pipetting, draw up MS water and expel to waste _____

        2. Dry exterior of piston _____

        3. Place tip of pipettor in sample (no more than 1 cm) and draw up and expel several times (avoid foam and bubbles) _____

        4. raw sample into pipettor _____

        5. Holding pipettor at about 90º to lab bench and with tip at about eye level, dry exterior of tip by quickly wiping from the pipettor over the tip _____

        6. Carefully inspect the pipettor tip to insure sample volume is flush with the tip _____

        7. If concave, re-sample _____

        8. If convex, re-wipe as above to achieve a flush sample volume (see item 13.c.3.a.5) _____

    b. For air displacement pipettor with new tip for each sample

        1. Depress plunger and place tip into sample (avoid foam or bubbles) _____

        2. Draw up test portion _____

        3. Remove from sample _____

        4. If excess product adheres to tip, wipe carefully without wicking sample _____

4. Dispel 75 µL (or 25 µL) of sample 1 cm below the surface of the calibrator (do NOT dispense down side of cuvette) _____

    a. With tip still below surface depress plunger three times into calibrator to completely expel sample _____

        1. With plunger still completely depressed, remove from tube _____

5. Mix immediately by vortexing, or mix by inversion after covering with Parafilm M or cap _____

6. Place cuvette in Fluorometer within 20 sec of adding product to calibrator _____

7. After each reading, remove cuvette and close door immediately _____

8. Maintain records of the calibrators _____

d. Re-calibration required if: _____

1. Controls out of limits _____

2. Adjustments made to bring A-D mode checks (item 11) into specification _____

3. Any significant instrument service if performed, e.g. lamp or filter replaced _____

e. Instrument checks and calibrations are within specification _____

CONTROLS

14. Negative Control _____

a. Use PhosphaCheck negative control from set in item 10.c _____

b. Or, optionally, prepare by heating a sample of product to 95±1ºC, stirring or mixing as necessary (TC used) _____

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

2. Use within 24 hours or aliquot 1 mL quantities within 24 hours 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: _____ _____

c. Test control as a sample (see item 16.b-k) _____

d. Value less than (<) 20 mU/L: _____ _____

e. Maintain records _____

15. Positive Control _____

a. Use PhosphaCheck positive control from set in item 10.c _____

b. Or, optionally to a portion of negative control (item 14.b), add approximately 0.1 mL of mixed-herd raw milk and bring up to approximately 100 mL with additional negative control _____

1. Use within 24 hours or, aliquot 1 mL quantities within 24 hours 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: _____ _____

c. Test control as a sample (see item 16.b-k) _____

d. Value between 500±150 mU/L _____ _____

e. Maintain records _____

TEST PROCEDURE

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

a. Perform all instrument and reagent checks (item 12), negative control test (item 14) and positive control test (item 15) prior to running analysis _____

b. Using reagent dispenser, fixed volume or electronic pipettor, or sterile serological pipet, dispense 2.0 mL of working substrate into labeled 12 x 75 mm glass cuvettes _____

1. Prime reagent dispenser (item 5) 3x prior to dispensing volumes to cuvettes to remove any bubbles from dispenser tubing _____

c. Warm substrate to 38±1ºC in the heating block for 20 min (use within 4 hours) _____

d. Select the product type channel and enter identification number _____

e. Sample agitation _____

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 subsamples of retail milk containers or controls by shaking 25 times in 7 sec with a 1 ft movement or vortex for 10 sec at maximum setting (subsamples in appropriate containers to allow the use of vortexing), use within 3 min _____

f. Add products to substrate one tube at a time just prior to being tested, remove test portions avoiding foam and bubbles _____

1. For positive displacement pipettors with reusable tip(s) _____

a. Prior to pipetting, draw up MS water and expel to waste _____

b. Dry exterior of piston _____

c. Place tip of pipettor in sample (no more than 1 cm) and draw up and expel several times _____

d. Draw sample into pipettor _____

e. Holding pipettor at about 90º to lab bench and with tip and at about eye level, dry exterior of tip by quickly wiping from the pipettor over the tip _____

f. Carefully inspect the pipettor tip to insure sample volume is flush with the tip _____

g. If concave, re-sample _____

h. If convex, re-wipe as above to achieve a flush sample volume (item 16.f.1.e) _____

2. For air displacement pipettor with new tip for each sample _____

a. Depress plunger and place tip into sample (avoid foam or bubbles) _____

b. Draw up test portion _____

c. Remove from sample, touch off to side of container _____

d. If excess product adheres to tip, wipe carefully without wicking sample _____

g. Dispel 75 µL (or 25 µL) of sample about 1 cm below the surface of the substrate (do not dispense down side of cuvette) _____

1. With tip still below surface depress plunger three times into substrate to completely expel sample _____

2. With plunger still completely depressed, remove from tube _____

h. Mix by vortexing, or by inversion after covering with Parafilm M or cap _____

i. Place cuvette in Fluorometer within 20 sec of adding product to substrate and close cuvette door _____

j. Results will display in 3 min, save tape printout of results and record in QC record _____

1. If a 25 µL sample volume was used multiply the displayed value by 3 _____

2. Record adjusted value in QC record _____

k. Samples with ≥350 mU/L or more of ALP activity are suspect positive and must be confirmed (item 17) _____

l. Maintain records _____

CONFIRMATION

17. Positive Confirmation _____

a. Retest suspect positive sample _____

b. Samples with ≥ 350 mU/L of ALP activity are suspect positive and must be tested for microbial, and reactivated phosphatase (items 18 & 19) _____

18. Negative Control _____

a. Prepare a negative control from each suspect product _____

b. For the preparation of control using the suspect product: _____

1. Prepare by heating sample for at least 1 min after temperature measuring device registers 95±1ºC, stirring or mixing as necessary (TC used) _____

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

c. Negative control must be less than 20 mU/L when tested _____

19. Microbial 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 rapidly to 0.0-4.5ºC in an ice bath _____

c. Test heated sample, unheated sample and negative controls _____

d. Interpretation _____

1. If heated and unheated samples have equal activity (within ±5%), the sample is regarded Not Found for residual phosphatase, the activity originally measured is microbial _____

2. If the heated portion has significantly reduced (>5%) or no activity, the sample contains milk phosphatase activity, either residual or reactivated _____

20. Reactivated Phosphatase _____

a. Magnesium acetate solution commercially available _____

b. Or, prepared in laboratory _____

1. Dissolve 35.4g of Mg(C2H302)2·4H20 in 25 mL MS 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, make up to 100 mL (stable for 1 year at 0.0-4.5ºC) _____

c. Procedure _____

1. Place 10 mL of each milk or milk product sample to be tested in a boiling water bath and hold 1 min after temperature sample has reached 95±1ºC (Use TC) _____

2. Cool samples rapidly to 0.0-4.5ºC in an ice bath _____

3. Place a 5 mL aliquot of sample (unheated) to be tested in a screw-cap test tube and add 0.1 mL MS water ("Blank" sample) _____

4. To a second 5 mL aliquot (unheated) in an identical tube, add 0.1 mL Mg acetate solution ("Test" sample) _____

5. Cap tubes and incubate both aliquots for 1 hour at 34±1ºC (Use TC) _____

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

7. Dilute 1 mL of sample containing Mg acetate (Test) with 5 mL (1:6 dilution) of corresponding boiled milk or milk product control (items 19.b.1 & 2 above) _____

8. Test undiluted sample containing no magnesium (Blank) and diluted sample containing Mg acetate (Test) for phosphatase activity (as described in item 16) _____

d. Interpretation _____

1. If the diluted aliquot containing Mg acetate (Test) has equal (±5%) or greater phosphatase activity than the undiluted aliquot containing no Mg acetate (Blank), the sample is regarded as negative for residual phosphatase, and the phosphatase originally measured is of **reactivated** origin _____

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

2. If the diluted aliquot (Test) contains less activity (< 5%) 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 (20ºC) for periods of 1 hour or more before testing (SPC <20,000/mL) _____

RECORDING, INTERPRETATION, AND REPORTING

21. Recording and Interpretation _____

a. Record values _____

b. Interpret _____

1. If value obtained is 349 mU/L or lower, sample is **Not Found (NF)** _____

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

22. Report _____

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

1. <350 mU/L _____

2. ≥350 mU/L but: _____

a. Meets reactivated phosphatase criteria (item 20.d.1) _____

b. Meets microbial phosphatase criteria (item 19.d.1) _____

c. Documentation shows the product was treated in such a way that reactivated phosphatase may be present _____

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

1. ≥350 mU/L and: _____

    a. Meets residual phosphatase criteria (item 20.d.2) _____

    b. No microbial phosphatase present (item 19.d.2) _____

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