

PHOSPHATASE TEST – NEOGEN ACCUPOINT ADVANCED ALKALINE PHOSPHATASE

IMS #

[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]

APPARATUS

2. **See CP items 1 - 32 (as necessary)** _____
- a. Unless otherwise states, “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. **AccuPoint Advanced Reader** _____
- a. Operating manual available _____
- b. Reader serial number: _____
- c. On-screen Product Definitions: _____
- 1. Whl Milk – Whole milk _____
 - 2. 2% milk – 2% milk fat _____
 - 3. 1% milk – 1% milk fat _____
 - 4. Skim – Skim milk _____
 - 5. LactFree – Lactose free or reduced milk (testing not required per M-a-98) _____
 - 6. StbryMlk – Strawberry milk (all fat levels) _____
 - 7. ChocMlk – Chocolate milk (all fat levels) _____
 - 8. HalfHalf – Half and Half _____
 - 9. Hvy Crm – Heavy Cream _____
4. **Pipettors and Pipets** _____
- a. Fixed volume or electronic, 1.0 mL _____
- b. Calibration checked as specified in CP item 6.e; maintain records _____
- c. Disposable, 10 mL (ASTM) pipet with 0.1 mL graduations _____
5. **Vortex Mixer** _____
6. **Water Bath, circulating, $34\pm 1^\circ\text{C}$ and $63\pm 1^\circ\text{C}$ (or $66\pm 1^\circ\text{C}$ if fat $>10\%$) (Confirmation Procedures)** _____

7. Test tubes (if lab runs Chocolate milk, Half and Half or Heavy Cream)

- a. Size appropriate for 1:1 dilution

8. Handling and Storage

- a. Kit contains Samplers (testing devices) and Lyophilized Positive Calibrators

Kit Lot # _____ Rcd. Date: _____ Exp. Date: _____

Lyophilized Calibrator Lot # _____ Exp. Date: _____

- b. Reagents stored at 0.0 - 4.5°C until expiration date

- c. Samplers (testing devices) must be at room temperature at time of use

CONTROLS

9. Negative Calibrator/Control

- a. Prepare at least 20 mL of negative sample for use as a Negative Calibrator/Control and to rehydrate the 350 mU/L Positive Calibrator

1. Heat a sample of product to 95-100°C for 2 min with stirring

- a. Cool rapidly in an ice bath and hold at 0.0-4.5°C

- b. Chocolate milk, Half and Half and Heavy Cream are diluted 1:1 with deionized (DI) or MS water prior to testing. All other products are tested undiluted

- c. Store at 0.0-4.5°C, the Negative Calibrator/Control may be used for up to 48 hours

- d. Or, aliquot 5.0 mL quantities into small tubes within 24 hours, seal and freeze at -15°C or colder in a non-frost-free freezer, or place in an insulated foam container in a frost-free freezer. Use within 2 months

Lab Prep. Date: _____ Lab Exp. Date: _____

1. Thaw at room temperature

2. Store at 0.0-4.5°C and use within 48 hours. Do not re-freeze

10. Positive 350 mU/L Calibrator

- a. Add 2.0 mL of Negative Calibrator/Control (item 9) to the calibrator vial. Cap the vial and vortex for 15 sec on high to solubilize the lyophilized material

- b. Hold at 0.0-4.5°C for 5 minutes

- c. Vortex again for 15 seconds _____
- d. Store are 0.0-4.5°C, the Positive Calibrator may be used for up to 48 hours _____

11. Positive Control _____

- a. For all products except Chocolate milk, Half and Half and Heavy Cream, the Positive Calibrator (item 10) is the Positive Control _____
- b. For Chocolate milk, Half and Half, and Heavy Cream the Positive Control is made by adding another 2.0 mL of Negative Calibrator/Control (item 9) to the 2.0 mL of Positive Calibrator (item 10), cap and vortex for 15 sec. [The 1:1 dilution is required to account for the dilution factor used for these products] _____

CALIBRATION

12. Calibrate reader with each new lot for each product type to be tested _____

- a. Prepare a Negative Calibrator/Control and a Positive Calibrator as described in items 9 and 10 _____
- b. Negative Calibrator Procedure _____
 - 1. Scroll to the line below Calibration that lists product definitions (item 3.c) _____
 - 2. Select the product to calibrate by pressing OK so the outline box turns red _____
 - 3. Navigate to the “product definition” 0-zero for the product type being calibrated and press the OK. The outline box turns blue (for example: Whl Milk 0 with a blue outline box) _____
 - 4. Test the control following test procedure (item 14.d - i) _____
- c. Positive Calibrator Procedure _____
 - 1. Scroll to the line below Calibration that lists product definitions (item 3c.) _____
 - 2. Select the product to calibrate by pressing OK. The outline box turns red _____
 - 3. Navigate to the “product definition” 350 for the product type being calibrated and press the OK. The outline box turns blue (for example: Whl Milk 350 with a blue outline box) _____
 - 4. Test the control following test procedure (item 14.d - i) _____
- d. Verify the Calibration _____
 - 1. Scroll the Reader to line labeled Calibration _____
 - 2. Press the OK button to change the outline box to red _____

3. Navigate to the line labeled Sample and press OK to change the outline box to blue _____
4. Scroll down to select the product that was just calibrated and press OK. The outline box is blue _____
5. Retest the Negative Calibrator/Control (item 9) following test procedure (item 14.d - i) _____
6. For all products except Chocolate Milk, Half-and-Half and Heavy Cream, retest the Positive Calibrator (item 10) following test procedure (item 14.d - i) _____
 1. For Chocolate Milk, Half-and-Half, and Heavy Cream add another 2.0 mL of Negative Calibrator/Control to the 2.0 mL of Positive Calibrator (item 10), cap and vortex for 15 sec. Test following the test procedure (item 14.d – i) [The 1:1 dilution is required to account for the dilution factor used for these products.] _____
7. Performance of negative must be <30 mU/L and positive must be in the range (250 – 500 mU/L) _____
8. If not within range, repeat starting from 12.b _____

DAILY PERFORMANCE CHECKS

13. Daily test of a Negative Control (item 9) and Positive Control (item 11) _____

- a. Test the Negative Calibrator/Control (item 9) following test procedure (item 14.b - i). Control must be ≤ 30 mU/L _____
- b. Test the Positive Control appropriate for the product type (item 11 a or b) following test procedure (item 14.b - i). Control must be 250 – 500 mU/L _____

TEST PROCEDURE

14. Test Procedure _____

- 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. For subsamples of retail milk containers or controls, mix by shaking 25 times in 7 sec with a 1 ft movement or vortex at least 15 sec at maximum setting; (subsamples in appropriate containers to allow the use of vortexing) use within 3 min _____

3. Chocolate milk, Half and Half and Heavy Cream are diluted 1:1 with deionized or MS water prior to testing

- a. Use a minimum of 10 mL of sample + 10 mL of water

 - b. Add 1.0 mL of diluted sample into a 2 mL centrifuge tube

- b. On the AccuPoint Advanced Alk Phos assay screen, scroll to the 2nd line and press the ok button. Navigate down to Sample and press the button so Sample with a blue outline box is shown

- c. Scroll to the 3rd line, press the ok button, scroll to the product description and press the ok button so the product with a blue outline box is shown

- d. Verify ALP sampler (testing device) stored at room temperature

- e. Pull the flocked handle from sampler (testing device) and submerge the flocked portion into the sample (item 14.a) or control (items 9 or 11). Swirl the flocked handle in the sample for 3 seconds and then place the flocked handle back in the sampler (testing device) and push the top of the sampler (testing device) handle until the flocked portion is submerged in sampler (testing device) reagent. Immediately shake vigorously for 10 seconds keeping the reagents in the tip of the sample

- f. Within 10 seconds, open the lid on the Reader by pressing the button on the left side of the reader. Insert sampler (testing device) into the reader and close the lid

- g. The reader will show a magnifying glass at the bottom of the screen and will collect data for the sample for the next 2 minutes. Do not open the reader lid or remove the sampler (testing device) during this time

- h. Near the end of the data collection period a red bar will advance on the screen and then the reading in RLU for calibrations and the reader converts the response to mU/L for samples which will be displayed on the screen

- i. Remove the sample, close reader door while not in use, and prepare for the next sample

- j. Samples with ≥ 350 mU/L of ALP activity are suspect positive and must be tested for microbial, and reactivated phosphatase (Sections 15 & 16)

CONFIRMATION

15. Microbial 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

1. If fat content is $>10\%$, heat at $66 \pm 1^\circ\text{C}$ for 30 min

- b. Cool sample rapidly to $0-4.5^\circ\text{C}$ in an ice bath

- c. Test positive and negative controls following item 13 _____
- 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 sample have equal activity ($\pm 30\%$, 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, $\text{Mg}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 4\text{H}_2\text{O}$ 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 rinse 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. Label separate test tubes as "Blank" and "Test" _____
 - 2. Add a 5.0 mL aliquot of sample (unheated, original sample prepared as in 14a) to each test tube _____
 - 3. Add 0.1 mL DI 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 $34 \pm 1^\circ\text{C}$ _____
 - 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 magnesium acetate (Test) with 5 mL (1:6 dilution) of negative control product (item 9), label tube as "Diluted Test" _____

7. Test undiluted sample containing no magnesium acetate (Blank) and diluted sample containing magnesium acetate (Diluted Test) for phosphatase activity following item 14

d. Interpretation

1. If the diluted aliquot containing Mg acetate (Diluted Test) has equal (30%) or greater phosphatase activity than the undiluted aliquot containing no Mg (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 hr or more before testing (SPC $<$ 20,000/mL)

RECORDING, INTERPRETATION, AND REPORTING

17. Record and Interpretation

a. Record values

b. Interpret

1. If value obtained is $<$ 350 mU/L the sample is **Not Detected**
2. If value obtained is \geq 350 mU/L the sample is **Actionable**

18. Report

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

1. Value is $<$ 350 mU/L
2. Value is \geq 350 mU/L but:
 - a. Meets reactivated phosphatase criteria (item 16.d.1)
 - b. Meet microbial/heat stable phosphatase criteria (item 15.e.2)
 - 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 16.d.2) _____
- b. No microbial phosphatase present (item 15.e.3) _____
- c. No documentation to show the product could have become reactivated _____