

## Alkaline Phosphatase (ALP) Test for Pasteurized Milk

The alkaline phosphatase (ALP) is an enzyme normally present in raw milk and it is inactivated in conditions of heat treatment. The temperature of inactivation of ALP is slightly higher than that required for the destruction of pathogenic bacteria. So the ALP test in pasteurized milk is used to verify if the heating process of pasteurization is done correctly. Recently, The U.S. and EU public health limit for alkaline phosphatase in pasteurized milk is below 350 mU/liter by using fluorescent detection method. So far, this method is the most effective and reliable method.

The assay uses specific enzyme-coupled reactions to produce fluorescent product. The fluorescence intensity at  $\text{ex/em} = 360/460\text{nm}$  is directly proportional to ALP concentration in the sample. The handheld fluorometer has internal temperature control, which controls the reaction at a constant temperature, allowing the system to provide reliable and rapid results within 20 minutes.

### Assay Performance:

- Sensitive & accurate, using 10  $\mu\text{L}$  /sampling.
- Linear detection range > 0 - 1,000 mU / L.
- Sensitivity: 20 mU/L, reaction time: 20 minutes.

### Content of the Field Kit:

- Handheld fluorometer (*Inno FluoroQuik*) · 360ex / 460em °
- ALP Assay Kit (50 tests)

### Content of the Assay Kit (50 tests):

- Reagent : 4.6 mL
- 100x Standard : 30  $\mu\text{L}$
- Mini Glass Tube : 50 pcs
- 0.5mL Plastic Vial : 5 pcs

### Other Materials Suggested:

- Transfer pipette, 10- $\mu\text{L}$  & 100- $\mu\text{L}$  Pipette Tip
- Timer
- Small incubator maintained at 38 ° C (for multiple sample measurements)

### Assay Procedure :

NOTE: Prior to assay, bring the assay reagents to room temperature. Switch on the fluorometer and set the temperature at 38 ° C.

**Calibration :** (For each new assay kit, a new calibration of the fluorometer is recommended. )

1. After the fluorometer reaches 38 ° C, pipette 90  $\mu\text{L}$  of reagent into a mini-glass tube and pre-heat for 5 minutes.
2. Prepare Standard: Mix 5  $\mu\text{L}$  of provided 100x Standard with 495 $\mu\text{L}$  of distilled water into a 0.5mL Plastic Vial to get 1x Standard.
3. From the main screen, press [Calibrate] → [Assay 1].
4. Place 10  $\mu\text{L}$  of 1x standard in a mini glass tube and stir the solution with a pipette tip to mix well. Put the glass tube back to the fluorometer. Heat for 30 - 60 seconds.
5. Press [Blank] to measure the "blank" tube, at the same time, set up the timer to stop after 20 minutes.
6. From the main screen, use the "<" and ">" arrow keys to set up the value "1000.00". When the timer reaches 20 minutes, press [Calibrate Std]. If the fluorometer shows "Calibration Finished", the fluorometer is now calibrated. Press [Return] to go back to the Main screen to start your sample measurement. The calibration remains in the fluorometer even when the power is off.

Therefore, no recalibration is required before using the new assay kit.

### Measurement :

1. Pipette 90  $\mu\text{L}$  of reagent into a mini-glass tube and pre-heat for 5 minutes.
2. Place 10  $\mu\text{L}$  of milk sample in a mini glass tube and stir the solution with a pipette tip to mix well. Put the glass tube back to the fluorometer. Heat for 30 - 60 seconds.
3. Press [Measure] → [Blank]. After the "blank" tube is measured, press [Measure] immediately and set up the timer to stop after 20 minutes or use the on-screen timer to wait for the next measurement. The value displayed on the screen should be near zero.
4. When the timer reaches 20 minutes, press [Return] → [Measure]. The concentration will be displayed in the window.
5. Record the data, or press [Save] to save the data in the on-board memory. Press [Return] and then [Measure] for the next sample.
6. If you want to measure multiple samples, use a small incubator to preheat multiple reagent-filled glass tubes.

### Product Information :

Shipping and storage: the kit is shipped at room temperature.

Store Reagents and Standards at -20°C. Shelf life: 12 months.

The handheld fluorometer is powered by the supplied 6V power adapter. No batteries available.

