

# EnzoTact<sup>®</sup> PRO

## Protease Detection Kit for Milk Samples

Art No: 10010 (96 tests)

Product nr.	Content	Product name
10010	96 tests	EnzoTact PRO Reagent Set
Contains:		
10007	2x 250 µg	EnzoTact PRO Dry Substrate
10008	2x 15 ml	EnzoTact PRO Substrate Buffer
10009	2x 11 ml	EnzoTact PRO Buffer A

### Description/ Intended Use:

EnzoTact<sup>®</sup>PRO is a rapid test method for the detection of proteases in milk samples. The Relative Fluorescence Units (RFU), emitted after contact with the target proteases, correspond with a concentration of these proteases. The detection reaction is measured using a specially designed small hand-held NIR fluorometer, the DeNIRO<sup>®</sup>

The two step test procedure requires a precipitation step and a 30 minute incubation period at room temperature.

The test is intended to be used by an analyst with experience in aseptic technique in a laboratory or other controlled environment facility.

### Application:

Proteases are produced by many bacteria and can still be present in milk after pasteurization. The presence of proteases in post-pasteurization milk can lead to sweet curdling of the milk. EnzoTact<sup>®</sup>PRO is designed to test post-pasteurization milk for the presence of residual Proteases.

### Required Materials (Not Provided):

Detact Diagnostics DeNIRO<sup>®</sup> NIR fluorometer  
MySPIN 12 minicentrifuge  
Vortex  
Pipette and tips for 0.2-1 mL and 20 µL volumes  
Eppendorf cups 2.0 mL and 0.5 mL  
Timer

### Test Procedure:

#### Step 1: Preparation of Substrate:

Pour all Substrate Buffer (15 mL) into the bottle containing Substrate and swirl gently mixing to dissolve all material.

*NOTE: Keep Substrate refrigerated (2–8 °C).*

*NOTE: At the beginning of every day, every afternoon and with a new batch of Substrate, a blank should be performed.*

To run a blank:

1. Pipette 300 µL of the Substrate to a 0.5 ml Eppendorf cup.
2. Insert the Eppendorf cup into the DeNIRO<sup>®</sup> NIR Fluorometer, close lid and follow instructions to run a blank. The results will appear after seconds.

#### Step 2: Sample preparation:

To run samples:

1. Pipette 1 mL of the milk sample directly into a 2ml Eppendorf tube.
2. Pipette 200 µL of Buffer A to the milk sample and vortex to mix thoroughly.
3. Let stand for exactly 5 minutes at room temperature.
4. Centrifuge tube for 5 minutes at 12,500 RPM.

#### Step 3: Detection of Protease:

1. Pipette 20 µL of the clear supernatant (through the fat-layer) and transfer to a 0.5 mL Eppendorf cup

2. Pipette 300 µL of the Substrate to the supernatant sample and vortex gently to mix the sample with the substrate.
3. Incubate for 30 (±1) minutes at room temperature.
4. Insert the Eppendorf cup into the DeNIRO<sup>®</sup> NIR Fluorometer, close lid and follow instructions to run a sample to initiate measurement. Results will appear after seconds and will be displayed in RFU (Relative Fluorescence Units). Refer to Interpretation of Results.

### Interpretation of Results:

The results are displayed in Relative Fluorescence Units (RFU). The table below shows the equivalent values of RFU measurements for concentrations of protease in a sample and is calibrated for Subtilisins. Since proteases will be measured as a total and may vary in the milk used, every facility should make its own reference table.

The RFU is given minus the background reading. Background RFU values must be established for each Substrate stock solution to be tested and will automatically be subtracted from readings by the DeNIRO<sup>®</sup> NIR fluorometer.

Concentration of Protease in sample (ng/mL)	RFU (DeNIRO <sup>®</sup> )
5	354
10	501
20	804
35	1276
55	1940
85	3011
100	3580
125	4578
300	13304

### Calibration Control:

It is advisable to run positive and negative controls according to Good Laboratory Practices.

### Storage & Shelf Life:

- Store reagents in a cool dark place (2–8°C).
- Do not use prepared Substrate after two days.
- Kits have a 6-months shelf life. Check the expiration date on label.

### Disposal:

All disposables should be disposed of safely in compliance with Good Laboratory Practice and Health and Safety Regulations.

### Safety & Precautions:

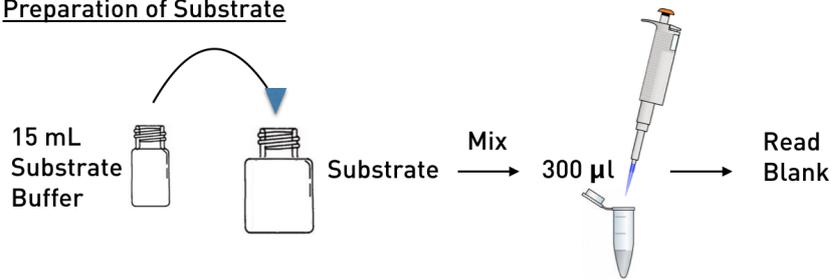
Components of EnzoTact<sup>®</sup>PRO do not pose any health risk when used correctly.  
Do not use reagents after Expiration Date.  
Sampling should be performed aseptically, to avoid cross contamination. Ensure proper incubation times and temperature.  
Place the DeNIRO<sup>®</sup> on a vibration-free surface (i.e. not on same table as vortex or centrifuge).  
The user must train personnel in proper handling of milk samples, testing techniques, and disposal.

### Detact Diagnostics Liability:

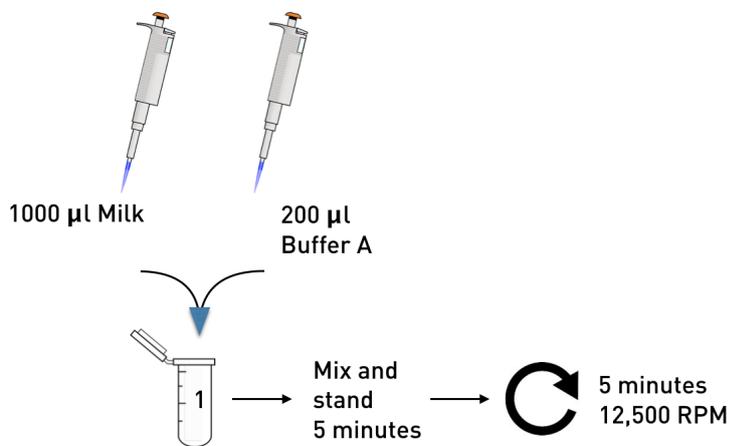
Detact Diagnostics will not be liable to user or others for any loss or damage whether direct or indirect, incidental or consequential from use of this test. If this product is proven to be defective, Detact Diagnostics' sole obligation will be to replace product or at its discretion, refund the purchase price. Promptly notify Detact Diagnostics within 5 days of discovery of any suspected defect and return product to Detact Diagnostics.

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## Preparation of Substrate



## Step 1: Sample preparation



## Step 2: Detection of Protease

