Psychrofast™ Test – Bulk Kit

Introduction

Rapid Detection of Psychrotrophic Bacteria in Dairy

Psychrotrophic bacteria, capable of thriving at refrigeration temperatures, are a primary cause of premature spoilage in pasteurized milk, significantly reducing its shelf-life. Conventional microbiological methods, such as aerobic plate counts and coliform testing fail to detect these contaminants. Aerobic counts primarily enumerate mesophilic bacteria, while coliform tests target enteric species, leaving psychrotrophic populations undetected. This oversight often results in product spoilage before the use-by date, even when standard tests indicate low microbial counts (<100 CFU/mL) and absence of coliforms (<1 CFU/mL). Existing dedicated methods for psychrotroph detection are impractical for routine quality control, requiring prolonged incubation periods of up to 10 days or, in modified protocols, 4 days.

The **Psychrofast Test** addresses these challenges by providing a rapid, reliable solution to detect gram-negative psychrotrophic bacteria in pasteurized milk and dairy products within **30 hours**. This simple presence/absence test employs selective enrichment technology, combining targeted inhibition of gram-positive bacteria with a visual growth indicator. By enabling timely detection, the test supports proactive quality management, helping to mitigate spoilage risks and optimize product shelf-life in the dairy industry.

Principle

The **Psychrofast Test** employs selective enrichment to detect gram-negative psychrotrophic bacteria in dairy products. The test utilizes two key components:

1. **Reagent A (benzalkonium chloride)**: Selectively suppresses gram-positive bacteria, eliminating interference from non-target organisms.
2. **Reagent B (tetrazolium chloride)**: A metabolic indicator that undergoes a redox reaction in the presence of actively growing bacteria.

During a 30-hour incubation at 30°C, psychrotrophic gram-negative bacteria metabolize nutrients, reducing the colourless tetrazolium chloride to formazan—a bright pink or red compound. A visible colour change to pink/red signifies a **positive result** (psychrotrophs present), while no colour change indicates a **negative result** (psychrotrophs absent). This method combines targeted inhibition with rapid visual detection, streamlining the identification of spoilage organisms in dairy samples.

Contents

- Reagent A – 750 tests (5% 2,3,5-Triphenyltetrazolium chloride) - sterile
- Reagent B – 750 tests (5% Benzalkonium chloride) - sterile

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Materials Required but not provided

- Incubator or waterbath set at (30 ± 1°C)
- 30ml sterile tubes / sample container
- Tube Rack
- 10ml pipet / pipettor
- UHT milk (negative control)
- Positive control (*Pseudomonas aeruginosa*)

Instructions for use

1. Mix the sample by shaking it up and down through an arc of 30cm 25 times in 12 seconds. If the headspace is insufficient for efficient mixing, aseptically pour the contents into a larger sterile container for further mixing
2. Pipet 10ml of sample into the sterile tube or container
3. For a blank control use 10mL of UHT milk into the tube
4. For positive control you can add 1 drop of raw milk into 10ml of UHT milk into the tube
5. Ensure aseptic techniques are used to minimize cross contamination
6. Add 2 drops (100ul) of Reagent A into the sample tube
7. Add 2 drops (100ul) of Reagent B into the sample tube
8. Incubate at 30°C ± 1°C for 30 hours

Interpretation

1. The presence of gram negative psychrotrops indicated by a pink coloration in the tube. The higher the levels the more intense the colors change.

Note: Larger volume increases the sensitivity of the test. Therefore, when 10mL of the sample are constantly producing negative results, the volume can be increased. This is a sign of improved quality of the product. Increasing sample size will also increase proportionally the levels of inhibitors added. For example, 15mL sample – add 3 drops (150ul mL) of both Reagent A and B. For every 5mL increase in sample size, the incubation time increases by ½ hour (30 minutes).

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Storage

Store in the fridge at 2–8°C. The Reagents A and B can be stored for up to 7 days at 25°C without any adverse effects.

Warranty

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