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Sarchomyces Cervisea Nucleic Acid Detection Kit (Fluorescent PCR Method)

Product Number: DTK813

Shipping and Storage

1. Store away from light at -20°C; shelf life is 12 months.
2. Low-temperature transportation shall not exceed 5 days; after opening, store away from light at -20°C with no impact on shelf life. Avoid repeated freeze-thaw cycles; up to 10 freeze-thaw cycles will not affect detection performance.

Component

Component	50T
Saccharomyces cerevisiae Primer Mix	100μL
Probe qPCR Amplification Mix	900μL
LactobacillusPositive control	100μL
Lactobacillus Negative control	100μL

Description

This kit is designed with specific primers and probes targeting highly conserved regions of the Saccharomyces cerevisiae genome. When the reaction system contains Saccharomyces cerevisiae genomic template, the PCR reaction proceeds and releases fluorescent signals. A real-time fluorescent quantitative PCR instrument is adopted to monitor and output signals of corresponding channels in real time during PCR, enabling qualitative analysis of detection results.

Application

Saccharomyces cerevisiae, also known as baker's yeast or budding yeast, is the yeast most widely associated with humans. Its cells are spherical or oval with a diameter of 5–10 μm, and it reproduces via budding.

Saccharomyces cerevisiae is widely applied in food, pharmaceutical and other industries. In the food industry, it is used for wine brewing, bread and pastry production; it also has certain applications in the pharmaceutical field. As a unicellular eukaryote, Saccharomyces cerevisiae possesses the fundamental characteristics of all eukaryotic cellular life activities, along with numerous advantages for experimental microorganisms including clear genetic background, fast growth rate and convenient operation.

Applicable instruments

ABI series, Bio-Rad series, Roche LC480, QIAGEN Rotor-Gene series, Tianlong Gentier-96E/96R series, Yaru MA6000, Hongshi SLAN-96S/96P and other multi-channel real-time fluorescent quantitative PCR instruments.

Specimen collection

1. Sample Selection: Saccharomyces cerevisiae samples can be collected from natural environments, specific production areas and industrial production processes. Research on these samples helps to understand the genetic diversity of yeast and its role in fermentation. Select appropriate samples for detection according to actual conditions during collection.
2. Aseptic Operation: Aseptic operation must be implemented during sample collection to avoid contamination by exogenous bacteria.
3. Storage: Samples can be stored at 2~8°C for no more than 72 hours; long-term storage is available below -70°C, but repeated freeze-thaw cycles shall be limited to a maximum of 5 times.

Protocol

1. **Sample processing (sample processing area)**

Commercially available kits are recommended for DNA/RNA extraction; strictly follow the instruction manual of the corresponding kit. Contamination shall be avoided during sample nucleic acid extraction.

2. **Reagent preparation (reagent preparation area)**

Calculate the required number of PCR reaction tubes as N based on the total number of samples to be tested (N = number of samples + 1 negative control tube + 1 positive control tube; prepare one extra reaction for every 10 samples). The reaction system for each test is formulated as shown in the table below:

Reagent	Volume
Saccharomyces cerevisiae Primer Mix	2μL
Probe qPCR Amplification Mix	18μL
Total Volume	20μL

3. **Sample addition (sample processing area)**

Transfer 5 μL each of the DNA/RNA extracted in Step 1, positive control and negative control into corresponding reaction tubes respectively, cap tightly, mix thoroughly and perform brief centrifugation.

4. **PCR amplification (nucleic acid amplification zone)**

4.1. Place the reaction tubes to be tested into the reaction slot of the real-time fluorescent quantitative PCR instrument;

4.2. Set channels and sample information; set the reaction system to 25μL; Fluorescence channel settings: Reporter Dye: FAM, Quencher Dye: NONE; DO NOT select ROX reference fluorescence;

4.3. Recommended loop parameter settings:

step	Cycles	Temperature	Time	Collect fluorescence signals
1	1 cycle	95℃	2min	No
2	40 cycles	95℃	10sec	No
		55℃	30sec	Yes

Note: For ABI series fluorescent PCR instruments, ROX correction shall not be selected during setup, and Quencher shall be set to None.

5. **Result analysis and judgment**

5.1. Kit validity judgment

Positive Control: Typical S-shaped amplification curve or Ct value ≤ 35.

Negative Control: Ct value > 38 or no Ct value, the curve is a straight line or slight slant without exponential growth phase..

5.2. Specimen result judgment

Positive: S-shaped amplification curve with Ct value ≤ 35.0 in detection channel and obvious exponential growth curve;

Suspicious: S-shaped amplification curve with Ct value ranging from 35.0 to 38 in detection channel. Retest is recommended. If Ct value remains between 35.0 and 38 with obvious growth curve after retest, judge as positive; otherwise negative.

Negative: Specimen Ct value > 38 or no Ct value detected.

Limitations of detection methods

1. False-negative results may occur when the concentration of target nucleic acid in the test sample is lower than the minimum detection limit of this kit.
2. Improper operation during sample collection, transportation, storage and processing may cause DNA/RNA degradation, leading to false-negative results.
3. Cross-contamination during sample collection, transportation, storage and processing may lead to false-positive results.

Performance

1. Minimum detection limit of the product: 300 Copies/mL;
2. Linear detection range: 300 ~ 1×10^{10} copies/mL;
3. Precision: Intra-assay and inter-assay coefficient of variation (CV) of precision reference samples ≤ 5%;

4. High specificity: No cross-reactivity with other pathogens that infect the same site or cause similar symptoms.

Note

1. All operations shall be carried out in strict accordance with the manual. Strict zoning operation shall be implemented for each stage of PCR to avoid cross-contamination.
2. To prevent DNA/RNA degradation, sample pretreatment shall be performed at 0–4°C, and samples shall be loaded onto the instrument immediately after pretreatment. All consumables used in sample pretreatment shall be DNase/RNase-free.
3. Negative and positive controls shall be set for each experiment.
4. All reagents of the kit shall be fully thawed and mixed at room temperature before use, followed by brief centrifugation.
5. All negative and positive controls equipped in the kit shall be transferred to the sample preparation area and stored separately before first use.
6. To avoid fluorescence interference, do not make direct contact with PCR reaction tubes, and do not make any marks on PCR reaction tubes.
7. Instrument amplification parameters shall be set in accordance with relevant requirements of this manual; reagents from different batches shall not be mixed.
8. Waste generated during the experiment shall be subjected to harmless treatment before disposal.