Candida Albicans Nucleic Acid Detection Kit Manual (Fluorescent PCR)

【Product name】

Candida Albicans Nucleic Acid Detection Kit (Fluorescent PCR)

【Packaging specification】

48 tests/box

【Intended use】

This kit is used for in vitro qualitative detection of candida albicans nucleic acid in clinical sputum and wound swab samples. Candida is a budding yeast-like fungus with positive Gram stain. It is an opportunistic pathogen. About 25% ~ 50% of healthy people have Candida in their oral cavity, vagina and digestive tract. When the body's immune function drops, candida can invade many parts of the body, causing skin candidiasis, mucosal candidiasis, visceral and central nervous candidiasis. Candida albicans is one of the main pathogenic bacteria in candida.

This kit is used for auxiliary diagnosis and curative effect monitoring of candida albicans infection.

【Detection principle】

This kit adopts polymerase chain reaction (PCR) and fluorescence probing technology to rapidly detect the specific genes (ERG11) of Candida albicans (hereinafter referred to as CA) in clinical samples, thereby judging the existence of Candida albicans. Uracil -N- glycosylation enzyme and dUTP are used in the kit to avoid contamination of amplification products.

【Kit contents】

Contents	Specification and quantity	Ingredients
10x concentration solution A	5 mL x 1 vial	NaOH etc.
10x concentration solution B	10 mL x 1 vial	Tris-HC 1 and EDTA
DNA extraction solution	5 mL x 1 vial	Surfactant (NP-40), TritonX-100
Extraction solids	48 tubes	/
CA-PCR reaction solution	1.1 mL x 2 vials	Buffer, probes, primers, $MgCl_2$, dNTP
Taq DNA polymerase (5U/μL)	25μL x 1 vial	/
Uracil N-Glycosylase (1U/μL)	10μL x 1 vial	/
Internal control	1 mL x 1 vial	Plasmids containing internal control
Negative control (CA)	1 mL x 1 vial	Tris-HC 1 and EDTA
Positive control (CA)	1 mL x 1 vial	Plasmids containing target gene

【Storage and validity】

Kit should be kept at -20±5℃ and it is valid for 8 months. Keep at 2-8℃ after opening and use within 1 week. Avoid repeated freezing and thawing (less than 5 times).

Transport the kit at low temperature. Use the ice packs for long distance transportation.

【Compatible instruments】

TIB-8600, ABI Prism 7500, STRATAGENE, Agilent Mx3000P

【Sample requirements】

1. Specimen types: sputum and wound swab samples.

2. Specimen collection:

2.1 Sputum

Sputum coughed from deep lung in the early morning is collected into 1-3mL sterile glass tube and sent for detection in a sealed condition. External use of respiratory drugs (ointments, sprays, drops) is prohibited within 24 hours before sampling.

2.2 Wound swabs

Sterile swabs collect secretion samples and then put them back into sterile swab sleeve for inspection. Samples shall be stored at room temperature for no more than 1 day and at 4-8℃ for no more than 6 days. External medicine (ointments, sprays, drops) are not allowed to be used within 24 hours before sample collection.

【Procedure】

1. Reagent preparation (reagent preparation area)

1.1 Dilute the 10× concentration solution A and 10× concentration solution B with sterile purified water at a volume ratio of 1: 9 to obtain cleaning solution A and cleaning solution B respectively, and place them in a refrigerator at 4℃ for later use.

1.2 Spin down Taq DNA Polymerase and Uracil NGlycosylase (UNG) and then place in a refrigerator at -20℃.

1.3 Confirm the number of reaction tubes n (n = sample number + negative and positive controls), and take out the CA-PCR reaction solution. Add n x 44.3μL CA-PCR reaction solution, n x 0.5μL Tag DNA Polymerase and n x 0.2μL URACIL N-glycosylase (UNG) into a centrifuge tube and vortex them evenly. After spinning down, sub-package 45μL into each PCR reaction tube and cover the tube and transfer to the sample adding area, and place in a 4℃ refrigerator.

1.4 Transfer the extraction solids, positive control, negative control and internal control to the sample processing area and place them in a refrigerator at 4℃.

2. Sample treatment

2.1 Sputum

2.1.1 Add 4 times volume of 1 x cleaning solution A into the glass tube. Shake and leave at room temperature for 15-30min to liquefy.

2.1.2 Transfer 1mL of liquefied specimen to 1.5mL centrifuge tube and centrifuge at 13000r/min for 5min.

2.1.3 Discard the supernatant, and add 1mL of cleaning solution B to the precipitate. Mix well and centrifuge at 13000r/min for 5min.

2.1.4 Discard the supernatant, and add 1mL of cleaning solution B to the precipitate. Mix well and centrifuge at 13000r/min for 5min.

2.1.5 Discard the supernatant and add 100μL DNA extraction solution to the precipitate for later use.

2.2 Wound swabs

2.2.1 Add 1mL of cleaning solution B (ensure that the cleaning solution can immerse above the sampling part of sterile swab), and vortex the sample tube at high speed for 2min to prepare a sample suspension.

2.2.2 Transfer all the suspension into a 1.5mL centrifuge tube, and centrifuge at 13000r/min for 5min then discard the supernatant.

2.2.3 Add 1mL of cleaning solution B and resuspend. Centrifuge at 13000r/min for 5min then discard the supernatant.

2.2.4 Add 100μL DNA extraction solution to resuspend the precipitate.

3. DNA extraction (sample treatment area)

3.1 Preparation of test samples

Add one tube of extraction solid to each of the above treated sample tubes (flick the bottom of the solids tube to pour the solid out as much as possible), and use a powerful vibrator (such as US BioSpec Mini-Beadbeater-16 or Vortex-Genie) to vortex for 5 minutes. Spin down and add 20μL of internal control.

3.2 Preparation of negative control

Centrifuge the negative control for several seconds at 8000r/min. Pipet 100μL to 1.5mL sterilized centrifuge tube, and add 20μL internal control.

Preparation of positive control Samples: (Same as Negative Control)

3.3 Spin down the test sample, positive control and negative control and dry bath at 95℃ for 2 minutes and ice bath 2-5 minutes immediately and then centrifuge at 13000r/min for 1min. Keep the supernatant for PCR amplification.

4. Sample adding (sample processing area or sample adding area)

Detection reaction: add 5μL of sample to be detected or negative control or positive control to the prepared PCR reaction tubes respectively, and spin down immediately after covering the tube tightly.

5. PCR amplification (Detection area)

Place the PCR tubes into the PCR instrument, edit the sample information and amplify as per below sequence:

Cycle Parameters:

Stage 1 37℃ ---- 2 min
Stage 2 94℃ ---- 2 min

Stage 3
10 cycles of
94℃ ---- 20 sec
55℃ ---- 45 sec
Stage 4
30 cycles of
94℃ ---- 20 sec
55℃ ---- 45 sec

CA Fluorescein detection: FAM
Internal control fluorescein: Texas Red
Reaction volume: 50μL
Fluorescent signal collection: stage 4, 55℃ --- 45 sec

【Reference Value】

Using the instrument matching software to implement automatically analysis, and obtain Ct number (FAM) for all the samples and controls.

<table>
<tr><td>1</td><td>Specimen (FAM) Ct value ≤ 23 with nice log amplification curve</td><td colspan="2">Positive</td></tr>
<tr><td>2</td><td>Specimen (FAM) Ct value = 30, or “No Ct” (Mx3000P) or “Undet” (ABI 7500), Internal Control (Texas Red) Ct value ＜ 30 with nice log amplification curve.</td><td colspan="2">Negative (below LOQ)</td></tr>
<tr><td rowspan="2">3</td><td rowspan="2">Specimen (FAM) 23 ＜Ct value ＜ 30</td><td rowspan="2">Vague result area, should be tested two more times</td><td>Retest twice, Ct value=30, negative</td></tr>
<tr><td>Retest twice, at least one Ct value ＜ 30 with nice log amplification curve, suspected positive, collect the specimen and test one more time or other detection methods suggested</td></tr>
</table>

【Results Interpretation】

1. Result analysis condition setting

1.1 ABI 7500 baseline setting: take the fluorescent signal line between cycle 2 and the sample cycle number 3 cycles before threshold is reached as the baseline. The threshold setting principle is that the threshold line just exceeds the peak of the normal negative control amplification curve, that is, Ct negative control = 30 or "Undet"

1.2 STRATAGENE Mx3000P baseline setting: select the fluorescence signal when "Adaptive baseline" is set. The threshold setting principle is that the threshold line just exceeds the peak of the normal negative control amplification curve, that is, Ct negative control = 30 or "No Ct"

2. Quality control standards

Positive and negative control should meet the following standards at the same time, otherwise the test is invalid.

2.1 Negative control, CA (FAM) Ct value = 30 or “No Ct” (Mx 3000p) or “Undet” (ABI 7500), internal control (Texas Red) Ct value ＜30.

2.2 Positive control, CA (FAM) Ct value ≤23 with nice log amplification curve, internal control (Texas Red) Ct value ≤30.

3. Results Interpretation

3.1 CA negative (lower than limit of quantitation): CA FAM Ct value = 30 or “No Ct” (Mx 3000p)

or “Undet” (ABI 7500), internal control (Texas Red) Ct value ＜30.

3.2 CA positive: CA (FAM) Ct value ≤23 with nice log amplification curve, internal control (Texas Red) Ct value ≤30.

3.3 Invalid results, retest is needed. CA FAM Ct sample = 30 or “No Ct” (Mx 3000p) or “Undet” (ABI 7500), internal control (Texas Red) Ct value = 30 or “No Ct” (Mx 3000p) or “Undet” (ABI 7500)

3.4 For samples with vague results: CA FAM 23 ＜ Ct sample ＜ 30, retests are suggested.

Note: Test result is for clinical reference only, and should not be taken as the only evidence to diagnose CA infection. Negative result only means that the DNA content is lower than the LOQ of this kit and should not exclude the possibility of CA infection. Further confirmation combining other clinical data is highly suggested for making a definite diagnosis when the result is positive.

【Detection Limitation】

This kit is suitable for clinical specimens detection, but the results are affected by the instruments and operation. Therefore, the results are for reference of clinical diagnosis only, and not the only criterion to confirm or exclude disease cases.

【Kit Performance】

The detection lower limit for this kit is 1.0×10^3copies/mL, with a linear range between 1.0×10^8copies/mL and 1.0×10^3copies/mL.

It is verified that this kit will not cross-react with other common clinical pathogens (group b streptococcus, staphylococcus aureus, staphylococcus epidermidis, enterococcus faecalis, streptococcus pneumoniae, micrococcus luteus, Bacillus cereus, streptococcus pyogenes, Escherichia coli, streptococcus bovis, Pseudomonas aeruginosa, candida parapsilosis, candida cruz, candida hilly, candida guilliermondii, candida sake, candida lactis, candida intermedia, cryptococcus laurentis, Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, candida glabrata and candida tropicalis)

The CV value within same batch and between different batches are both lower than 10%.

In the detection of clinical specimens of Candida albicans, the coincidence rate of this kit compared with bacterial culture and automated microbial identification system is above 95%, which meets the requirements of clinical application.

【Warnings and Precaution】

1. For in vitro diagnosis use only.
2. Read this manual in detail before the assay, and the assay should be carried out by skilled personnel.
3. Use latex gloves or thin film gloves when handling the PCR tubes.
4. Avoid unnecessary repeated freezing and thawing and keep the PCR solution away from lights.
5. Thoroughly thaw the reagents and spin them down briefly before using.
6. Sterilize centrifuge tubes and pipet tips in high temperature and high pressure before being used.
7. Processing and handling of clinical specimens should be carried out in a biosafety cabinet.
8. After being spin down, the PCR tubes should avoid vortex when being loaded on the PCR

instrument.

9. Avoid touching the extraction solids when aspirate DNA temperate.
10. Paraffin is suggested for sealing and cover the tube caps tightly after sample adding.
11. Dispose the PCR tubes in sealing airtight plastic bags as biohazard waste after the PCR instrument cool down at room temperature.
12. Dispose the pipet tips into the 10% sodium hypochlorite wasted solution vat and sterilize with other wastes.
13. Sterilize the biohazard safety cabinet by UV lights. After the experiment, clean the biohazard cabinet and pipets with 10% pasteurization, then use 75% ethyl alcohol for cleaning after 10 min.
14. Do not mix-use the reagents from different batches. Use this kit before its expiration.
15. Please strictly divide the laboratory into regions according to the principle of regional setting of clinical gene amplification testing laboratory of the Ministry of Health: reagent preparation room, sample preparation room and nucleic acid amplification room.

【References】

Guidelines for Writing Instructions for In Vitro Diagnostic Reagents, SFDA Released.

【Manufacturer】

Name of registered manufacturer: Triplex International Biosciences (China) Co., Ltd.
Residence: Unit 407, 408, and 409, 4th floor of Siming photoelectric building, 55 QianPu Industrial Park, Siming District, Xiamen.
Production address: Unit 101, 201, and 301 No. 2041, Unit 101, 201, and 501 No. 2045 Xizhou Road, Xike Town, Tongan District, Xiamen.
Zipcode: 361100 Tel: +86 592-3737666 Fax: +86 592-3788599
Enquiry Hotline: 400-7032032
Website: www.tibchina.com