



Dynamiker Biotechnology (Tianjin) Co., Ltd.

Dynamiker *Candida albicans* IgG Assay

Catalogue No.: DNK-1409-1

User Manual / 96 tests

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1. INTENDED USE

Dynamiker *Candida albicans* IgG Assay is based on indirect Enzyme-linked Immunosorbent Assay (ELISA). It is used for the quantitative detection of *Candida albicans* anti-mannan IgG antibodies in human serum or plasma, offering a diagnostic reference for *Candida albicans* infection. The kit is intended for professional use only.

2. PRINCIPLE

The diluted serum/plasma samples are pipetted into the wells coated with mannan antigen and incubate. Specific antibodies in the sample can bind to the immobilized antigens. After removing the unbound material by washing, the conjugate is added to each well and incubate to form immune complex. The substrate solution is added and incubate after further washing. Then the stopping solution is added to terminate the color development. The result is measured at 450 nm using an ELISA microplate reader. The intensity of color development is proportional to the concentration of IgG-specific antibodies detected.

3. SUMMARY AND EXPLANATION

Candida albicans is an opportunistic pathogen that inhabits the skin, mouth, gastrointestinal tract and vagina. It is the most commonly-isolated yeast in invasive *candidiasis* (IC) and occupies more than 65% of IC ^[1]. *Candida albicans* can adhere to host tissues, produce secretory aspartyl proteases and phospholipase enzymes, and transform from yeast to hyphal phase, which is the major determinant of its pathogenicity. Risk factors for infections with *Candida albicans* include immunosuppression prior to steroid use, leukocytosis, intensive care unit stays, or presence of intravascular or urinary catheters.

Candida species are the fourth most common cause of all nosocomial bloodstream infections (BSIs). Nearly 50% of mortality is attributable to such infections ^[2]. Appropriate initial antimicrobial therapy plays critical role in IC management and delay in the initiation of therapy would significantly increase the mortality of IC. The presence of IgG antibodies against *Candida albicans* indicates prior *Candida albicans* infection and suggests immunity. A significant rise of IgG levels indicates an acute infection or a re-infection.

4. KIT COMPONENTS

No.	Component	Content	Quantity
R1	Microtiter Strips	12 breakable strips with 8 wells each; coated with <i>Candida albicans</i> mannan antigen	1 plate / 12×8 wells
R2a	Standard a (500 AU/mL)	<i>Candida albicans</i> IgG antibodies in PBS with protein; Preservative: 0.05% ProClin300	1×1mL
R2b	Standard b (250 AU/mL)	<i>Candida albicans</i> IgG antibodies in PBS with protein; Preservative: 0.05% ProClin300	1×1mL



R2c	Standard c (125 AU/mL)	<i>Candida albicans</i> IgG antibodies in PBS with protein; Preservative: 0.05% ProClin300	1×1mL
R2d	Standard d (62.5 AU/mL)	<i>Candida albicans</i> IgG antibodies in PBS with protein; Preservative: 0.05% ProClin300	1×1mL
R2e	Standard e (31.25 AU/mL)	<i>Candida albicans</i> IgG antibodies in PBS with protein; Preservative: 0.05% ProClin300	1×1mL
R3	Conjugate	Rabbit-anti-human IgG antibodies, conjugated with HRP; stabilized with protein stabilization solution	1×12mL
R4	Concentrated Washing Solution (20×)	PBS and Tween 20; Preservative: 0.05% ProClin300	1×20mL
R5	Sample Dilution Solution	PBS with protein and Tween 20; Preservative: 0.05% ProClin300	2×50mL
R6	Substrate Solution	Tetramethylbenzidine (TMB)	1×12mL
R7	Stopping Solution	2M H ₂ SO ₄	1×8mL
R8	Control A	<i>Candida albicans</i> IgG antibodies dissolved in PBS with protein; Preservative: 0.05% ProClin300 Concentration: 125-250 AU/mL	1×1mL
R9	Control B	<i>Candida albicans</i> IgG antibodies dissolved in PBS with protein; Preservative: 0.05% ProClin300 Concentration: 31.25-60 AU/mL	1×1mL
M1	Plate Sealer	Adhesive membrane of microtiter plate	1× sheet

5. STORAGE AND STABILITY

Store unopened kit at 2~8°C. Once opened, follow the instructions below:

Item	Storage	Stability
Microtiter Strips coated with <i>Candida albicans</i> manan antigen	After opening, store in the sealed bag with desiccant at 2~8°C	4 weeks
Standards (a, b, c, d and e)	After opening, stored at 2~8°C	4 weeks
Conjugate	After opening, stored at 2~8°C	until expiry date
Concentrated Washing Solution	After opening, store the concentrated solution (20×) at 2~8°C	until expiry date



	After dilution, store the washing solution at 2-30°C	2 weeks
Sample Dilution Solution	After opening, store at 2~8°C	until expiry date
Substrate Solution	After opening, store at 2~8°C in dark	until expiry date
Stopping Solution	After opening, store at 2~30°C	until expiry date
Controls (A and B)	After opening, store at 2~8°C	4 weeks

6. MATERIALS NEEDED BUT NOT SUPPLIED

- 6.1 ddH₂O: for dilution of concentrated washing solution
- 6.2 Absorbent paper
- 6.3 Disposable gloves
- 6.4 Pipette tips (10µL, 50µL, 100µL, 1000µL)
- 6.5 Polypropylene centrifuge tubes (1.5mL, sealed and gas-tight)
- 6.6 Vortex mixer
- 6.7 Water bath
- 6.8 Incubator
- 6.9 Semi-automatic plate washer (Recommended)
- 6.10 Microplate reader and microplate shaker

7. SAMPLE COLLECTION AND STORAGE

Make sure the sample is not contaminated by fungal spores and bacteria. The sample must be placed in sealed tubes to avoid exposure to air during the process of transfer and storage. For long time storage, store the serum or plasma below -20°C. Avoid repeated freezing and thawing. Serum or plasma samples can be stored at 2-8°C for up to 5 days before testing.

8. FLOW CHART OF TESTING PROCEDURE

Samples pretreatment:

Add 1µL of samples into 1000µL of sample dilution and mix thoroughly



Addition of standards / controls / samples:

Add 100µL of the standards (a, b, c, d and e), controls and diluted samples into wells

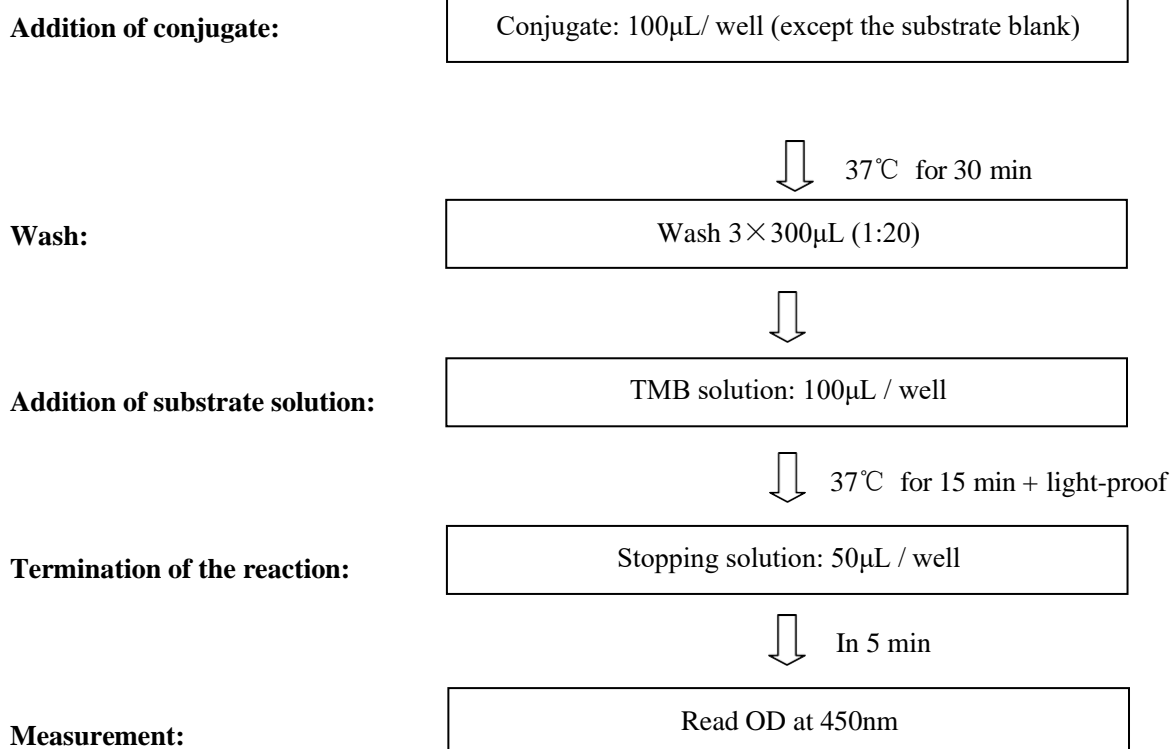


37°C for 60 min

Wash:

Wash 3 × 300µL (1:20)





9. PROCEDURE

- 9.1 Place all the reagents at room temperature (20-25°C) for 30 min before test.
- 9.2 Take the microtiter strips R1 out of the sealed bag. Place back the unused strips and seal the bag tightly and store it at 2-8°C.
- 9.3 Prepare washing solution:
Dilute the concentrated washing solution (20 \times) R4 with ddH₂O (e.g. 1mL R4 + 19mL ddH₂O). The diluted washing solution can be stored at 2~8°C for up to 2 weeks. Adequate washing solution should be prepared for the entire test.
- 9.4 Sample dilution solution:
Add 1 μ L of sample to 999 μ L of Sample Dilution solution R5. Mix well by vortex.
- 9.5 Leave one well for a substrate blank.
- 9.6 Pipette 100 μ L of the standards, controls and diluted samples into wells as below.

Wells	1	2
A	Substrate Blank	Sample 1
B	Standard a	Sample 2
C	Standard b	...
D	Standard c	...
E	Standard d	...
F	Standard e	...
G	Control A	...
H	Control B	...

- 9.7 Seal the microtiter plate with a plate sealer and incubate for 60 min at 37°C.



- 9.8 Remove the plate sealer and shake out the incubation solution. Wash the wells 3 times with at least 300 μ L/ well washing solution each time. The soak time is 40 seconds. After the last wash, invert the microtiter plate and remove all the remaining liquid by tapping on absorbent paper.
- 9.9 Add 100 μ L of conjugate into each well except the substrate blank.
- 9.10 Seal the microtiter plate with a plate sealer and incubate at 37°C for 30 min.
- 9.11 Repeat **step 9.8**.
- 9.12 Add 100 μ L of substrate solution into each well including the substrate blank.
- 9.13 Seal with a plate sealer and incubate it at 37°C for 15 min.
- 9.14 Add 50 μ L of stopping solution into each well in the same order and at the same speed of the substrate solution addition. Shake the microtiter plate gently to mix.
- 9.15 Read OD at 450nm within 5 min after addition of the stopping solution.

10. DATA ANALYSIS

The standard curve is displayed between concentration of IgG anti-mannan antibody as X-axis (logarithmic scale) and optical density as Y-axis (linear scale). The standard curve is plotted by a curvilinear regression. Determine the concentration of IgG anti-mannan antibody in serum and plasma samples against the standard curve.

11. QUALITY CONTROL

- Substrate Blank: the OD must be < 0.2 ;
 - Control A: The concentration must be within 125-250 AU/mL;
 - Control B: The concentration must be within 31.25-60 AU/mL;
- If these criteria are unmet, the test needs to be re-performed.

12. INTERPRETATION OF RESULTS

1. Concentration of IgG anti-mannan antibody < 80 AU/mL indicates a negative result.
2. Concentration of IgG anti-mannan antibody ≥ 120 AU/mL indicates a positive result.
3. $80 \text{ AU/mL} \leq$ Concentration of IgG anti-mannan antibody $< 120 \text{ AU/mL}$ indicates an inconclusive result. Additional sampling and testing within a week are recommended.

Note:

1. When the concentration IgG anti-mannan antibody is beyond the range of the standard curve:
 - OD_{sample} $<$ Standard R2e, it indicates a negative result.
 - OD_{sample} $>$ Standard R2a, it indicates a positive result. The sample is recommended being diluted and retested.

13. PRECAUTIONS

- 13.1 Prevent samples and reagents from contamination of fungi and bacteria.
- 13.2 Use a separate micropipette or individual disposable tips to avoid carry-over and cross-contaminations.
- 13.3 Use reagents with the same lot.
- 13.4 Chemical reagents (acid or alkaline) or dusts may affect the activity of conjugate.
- 13.5 While washing, all the wells are filled with the same volume of washing solution. After the last wash, invert the microtiter plate to dry it by tapping against the absorbent paper to ensure no washing solution left and no foam existing.



13.6 Keep the substrate solution away from strong light and avoid contacting with oxidant. The substrate solution is invalid when turning from colorless to light blue.

14. LIMITATIONS

The IgG antibody may not be detectable in some immunosuppressive patients.

15. WARNINGS

15.1 Don't pipette by mouth.

15.2 Don't smoke, eat or drink in areas where samples or reagents are handled.

15.3 Wear disposable gloves, laboratory coat and safety glasses when handling the reagents and patients samples. Wash hands thoroughly after testing.

15.4 All the used samples or consumptive materials must be treated as infectious medical waste.

15.5 The stopping solution is caustic and easy to cause ambustion. Please wear safety glasses, disposable gloves and laboratory coat during the test.

16. REFERENCES

- [1] M. A. Pfaller, D. J. Diekema. Epidemiology of Invasive Candidiasis: a Persistent Public Health Problem. *Clinical Microbiology Reviews*, 2007, p133-163
- [2] Kevin W. Garey, Milind Rege et al. Time to Initiation of Fluconazole Therapy Impacts Mortality in Patients with Candidemia: A Multi-Institutional Study. *Clinical Infectious Diseases*. 2006, 43: 25-31.



17. MANUFACTURER

Company Name: Dynamiker Biotechnology (Tianjin) Co., Ltd.










Registration Address: No.14 Building, Eco-Business Park, 2018 Zhongtian Avenue, Sino-Singapore Tianjin Eco-City, Tianjin 300467, China



Company Name: Lotus NL B.V.

Address: Koningin Julianaplein 10, 1e Verd, 2595AA, The Hague, Netherlands.

[SYMBOLS USED]

Symbol	Description
	Use By
	Batch Code
	Manufacturer
	Keep Away from Sunlight
	Temperature Limitation
	In Vitro Diagnostic Medical Device
	Authorized Representative in the European Community
	CE Mark
	Date of manufacture

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