Dynamiker Biotechnology (Tianjin) Co., Ltd.

Dynamiker *Candida* Mannan Assay

Catalogue No.: DNK-1403-1

User Manual / 96 tests

CONTENTS

1. INTENDED USE

Dynamiker Candida Mannan Assay is based on competitive Enzyme-linked Immunosorbent Assay (ELISA). It is used for quantitative detection of Candida mannan antigen in human serum, offering a diagnostic reference for Candida infection. The kit is intended for professional use only.

2. PRINCIPLE

The conjugate is made from highly affinitive and specific anti-mannan antibodies tagged with HRP. The pretreated serum is pipetted into wells coated with mannan antigen. Then the conjugate is added into each well. The antigen in serum competes with the coated antigen to bind to the conjugate. The plate is incubated to form immune complex. After removing the unbound material by washing, the substrate solution is pipetted and incubated. Then the stopping solution is added to terminate the color development. The result is measured at 450nm using an ELISA microplate reader.

3. SUMMARY AND EXPLANATION

With the wide application of broad-spectrum antibiotics, corticosteroid, immunosuppressant, anti-tumor drugs, as well as the prevalence of AIDS and the development of organ transplantation, Invasive Fungal Diseases (IFD) cases are increasing and becoming more complicated. Candida species are the fourth most common cause of all nosocomial bloodstream infections (BSI). Nearly 50% of mortality is attributed to such infections [1]. Appropriate initial antimicrobial therapy plays critical role in Invasive Candidiasis (IC) management and delay in the initiation of therapy would significantly increase the mortality of IC. The presence of mannan antigen against Candida indicates a prior Candida infection.

4. KIT COMPONENTS

No.	Component	Content	Quantity
R1	Microtiter Strips	12 breakable strips with 8 wells each; coated with *Candida* mannan antigen	1plate/ 12×8wells
R2a	Standard a (0.25 μg/L)	Mannan antigen in PBS with protein; Preservative: 0.05% ProClin300	1×1mL
R2b	Standard b (0.5 μg/L)	Mannan antigen in PBS with protein; Preservative: 0.05% ProClin300	1×1mL
R2c	Standard c (1.0 μg/L)	Mannan antigen in PBS with protein; Preservative: 0.05% ProClin300	1×1mL
R2d	Standard d (2.5 μg/L)	Mannan antigen in PBS with protein; Preservative: 0.05% ProClin300	1×1mL
R2e	Standard e (5.0 μg/L)	Mannan antigen in PBS with protein; Preservative: 0.05% ProClin300	1×1mL

R3	Conjugate	Anti-mannan antibodies, conjugated with HRP; stabilized with protein stabilization solution	1×8mL
R4	Sample Treatment Solution	EDTA Solution	1×10mL
R5	Concentrated Washing Solution (20×)	PBS with protein and Tween 20 Preservative: 0.05% ProClin300	1×12mL
R6	Sample Dilution Solution	PBS with protein and Tween 20 Preservative: 0.05% ProClin300	1×5mL
R7	Substrate Solution	Tetramethylbenzidine (TMB)	1×12mL
R8	Stopping Solution	2M H_2SO_4	1×8mL
R9	Control A	Mannan antigen in PBS with protein; 2.5-5.0μg/L	1×1mL
R10	Control B	Mannan antigen in PBS with protein; <0.5μg/L	1×1mL
M1	Plate Sealer	Adhesive membrane of microtiter plate	2 sheets

5. STORAGE AND STABILITY

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

Item	Storage	Stability
Microtiter Strips coated with *Candida* mannan antigen	After opening, store in the sealed bag with desiccant at 2~8℃	4 weeks
Standards (a, b, c, d and e)	After opening, store at 2~8℃	4 weeks
Controls (A and B)	After opening, store at 2~8℃	4 weeks
Conjugate	After opening, store at 2~8℃	until expiry date
Sample Treatment Solution	After opening, store at 2~8℃	until expiry date
Concentrated Washing Solution	After opening, store the concentrated solution (20×) at 2~8℃	until expiry date
	After dilution, store the washing solution at 2~30℃	2 weeks
Sample Dilution Solution	After opening, store at 2~8℃	until expiry date
Substrate Solution	After opening, store at 2~8℃ in dark	until expiry date
Stopping Solution	After opening, store at 2~30℃	until expiry date

6. WARNINGS FOR USERS

6.1. For in vitro diagnostic use.

6.2. For professional use only.
6.3. Do not pipette by mouth.
6.4. Use of this test kit with samples other than human serum is not recommended.
6.5. Wear protective clothing, including lab coat, eye/face protection and disposable gloves (synthetic, non-latex gloves are recommended) and handle the kit reagents and patient samples with the requisite Good Laboratory Practices. Wash hands thoroughly after performing the test.
6.6. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
6.7. Avoid splashing samples or solutions.
6.8. Biological spills not containing acid should be wiped thoroughly with an effective disinfectant. Disinfectants that can be used include (but are not limited to) a solution of 10% bleach (0.5% solution of sodium hypochlorite), 70% ethanol. Materials used to wipe up spills may require biohazardous waste disposal.
CAUTION: Do not place solutions containing bleach in the autoclave.
6.9. Spills containing acid should be appropriately absorbed (wiped up) or neutralized with sodium bicarbonate, and the area rinsed and wiped dry; if it contained biohazardous material, wipe the area with one of the chemical disinfectants.
6.10. Dispose of all specimens and materials used to perform the test as though they contain an infectious agent. Laboratory chemical and biohazardous wastes must be handled and discarded in accordance with all local, regional and national regulations.

7. PRECAUTIONS FOR USERS

7.1. FROZEN SERUM SAMPLES STORED IN UNKNOWN CONDITIONS MAY GIVE INACCURATE RESULTS DUE TO CONTAMINATION WITH FUNGUS AND/OR BACTERIA.
7.2. Do not use kit or any kit reagents after the stated expiration date.
7.3. Do not mix reagents from other kits that have different lot numbers.
7.4. Bring all reagents to room temperature for at least 30 minutes before use.
7.5. Mix thoroughly every reagent before use.
7.6. Mix thoroughly the Concentrated Washing Solution (R5) before preparing the Working Washing Solution, exercising care to avoid microbial contamination.
7.7. Do not conduct the test in the presence of reactive vapors (acids, alkalis, aldehydes) or dust, which could affect the enzymatic activity of the Conjugate.
7.8. For manual pipetting of controls and specimens, use individual pipette tips to prevent carryover of samples.
7.9. To ensure adequate washing of the wells, comply with the recommended number of wash cycles and ensure that all wells are completely filled and soak 40 seconds, then completely emptied. Washing should not be performed manually with a squeeze bottle.
7.10. Do not allow the microplate to dry between the end of the wash cycle and addition of reagents.
7.11. Do not use the same container for the Conjugate and Substrate Solution.
7.12. Do not allow Conjugate or Substrate Solution to come into contact with metal or metallic ions.
7.13. Avoid exposing the Substrate Solution to strong light during storage or incubation. Do not

allow the substrate solutions to come into contact with an oxidizing agent.

7.14. Avoid contact of the Stopping Solution with any oxidizing agent. Do not allow the Stopping Solution to come into contact with metal or metallic ions.

7.15. Do not pour any unused Conjugate back into the original container.

7.16. The Substrate TMB Solution must be colorless. The appearance of a blue color indicates the reagent is contaminated and should not be used.

8. MATERIALS NEEDED BUT NOT SUPPLIED

8.1 ddH2O: for the dilution of concentrated washing solution
8.2 Absorbent paper
8.3 Disposable gloves
8.4 Pipette tips (200μL, 300μL, 1000μL)
8.5 Polypropylene centrifuge tubes (0.6mL or 1.5mL, sealed and gas-tight)
8.6 Vortex mixer
8.7 Water bath or Heat block
8.8 Incubator
8.9 Semi-automatic plate washer (Recommended)
8.10 Microplate reader and microplate shaker

9. SAMPLE COLLECTION AND STORAGE

This test is performed on serum.

Collect blood samples according to standard laboratory procedures. Serum samples must be uncontaminated with fungal spores and/or bacteria. Transport and store samples in sealed tubes, unexposed to air. After initial opening, samples may be stored at 2-8°C for 48 hours prior to testing. For longer storage, store the serum at -20°C or less.

Avoid repeated freezing and thawing.

10. FLOW CHART OF TESTING PROCEDURE

Samples pretreatment:

300μL of serum + 100μL of sample treatment solution

⇩ Vortex for 10 sec

⇩ 100℃ for 3 min

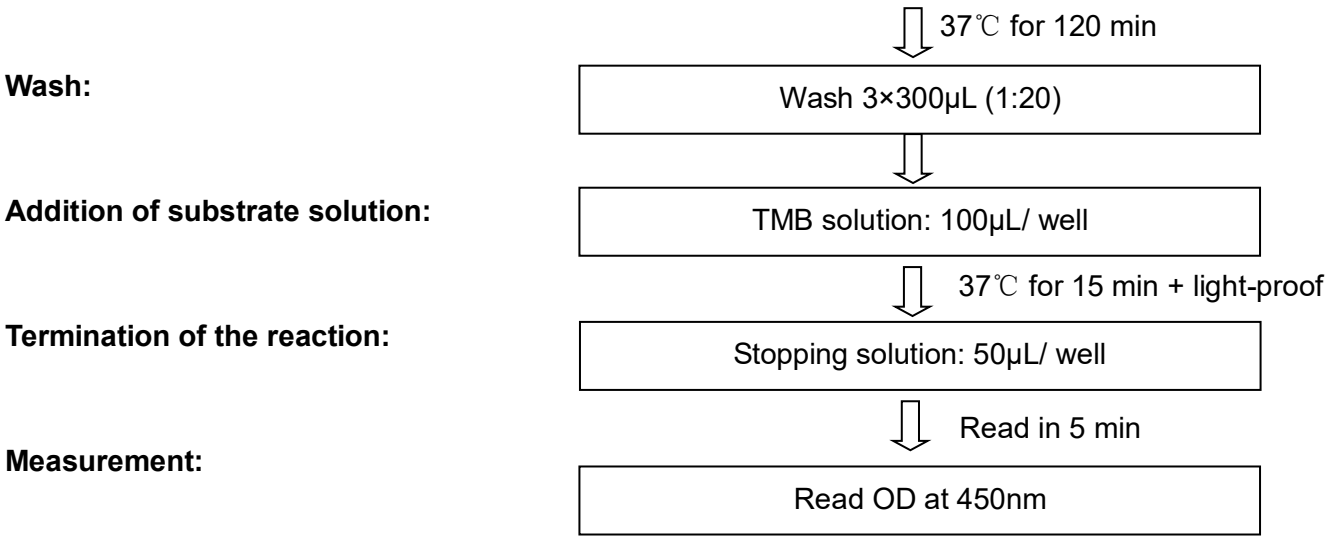
⇩ 10,000 × g for 10 min at 4℃

Addition of sample/ standards/ controls: Add 50μL of supernatant/ standards/ controls into microtiter strips

⇩

Addition of conjugate: Add 50μL of conjugate into microtiter strips

⇩ Shake the plate for 10 sec



11. SAMPLE TREATMENT

11.1 Pipette 300μL of serum into the centrifuge tube.

11.2 Pipette 100μL of sample treatment solution (R4) into each tube.

11.3 Vortex the centrifuge tube for 10 sec. Heat the tube at 100℃ for 3 min in water bath. Tightly close the tube to prevent opening during heating.

11.4 **Water bath option:**

If using a boiling water bath: heat tubes for 3 minutes at 100°C. Tubes must be placed in the water bath only when the prescribed temperature is reached.

11.5 **Heat block option:**

Heat tubes for 3 minutes in a heat block at 100°C. Tubes must be placed in the block only when the prescribed temperature is reached. The heat block should be opened at least 20 min before using to make the temperature stable. The size of centrifuge tubes should fit well with the well in the heat block. Do not rely on the temperature displayed by the apparatus, please check that the temperature complies with specifications by using a calibrated thermometer which will be fitted into a tube containing mineral oil: 100°C must be reached inside the tube in a heat block.

11.6 Centrifuge the heated tube for 10 min at 10,000×g at 4 ℃.

11.7 Collect 50μL of supernatant for detection.

12. ELISA PROCEDURE

12.1 Bring all reagents under room temperature (20-25℃) for 30 min before test. Put the microtiter strips(R1) back to the refrigerator and take out after sample treatment.

12.2 Take the microtiter strips out of the sealed bag (R1). Return the unused strips and reseal the pouch tightly, stored at 2-8℃.

12.3 Prepare washing solution:

Dilute the concentrated washing solution (20×) at 1:20 ratio with ddH2O (e.g. 1mL conc.

washing solution + 19mL ddH2O). The resultant washing solution is stored at 2~30℃ for up to 2 weeks. Adequate washing solution should be prepared for the entire test.

12.4 Add 50μL of standards (a, b, c, d and e), Controls (A and B) and samples into each well of the microtiter strips coated with Candida mannan antigen separately, and then add 50μL of Conjugate (R3) into each well. Do not add the Conjugate (R3) before the standards, controls and samples. Add 100μL of sample dilution solution (R6) into one well as the substrate blank.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Substrate blank	Sample 1	Sample 9									
B	Standard A	Sample 2	……									
C	Standard B	Sample 3										
D	Standard C	Sample 4										
E	Standard D	Sample 5										
F	Standard E	Sample 6										
G	Control A	Sample 7										
H	Control B	Sample 8										

12.5 Shake the plate well for 10 sec. Cover the microtiter plate with a plate sealer and incubate it at 37℃ for 120 min.

12.6 Remove the plate sealer and aspirate the incubation solution. Wash the wells 3 times with 300μL/ well washing solution each time. The soak time is 40 sec. After each wash, invert the microtiter plate and dry it by tapping on the absorbent paper.

12.7 Add 100μL of substrate solution (R7) into each well including the substrate blank.

12.8 Incubate the microtiter plate at 37℃ for 15 min without sealing.

12.9 Add 50μL of stopping solution (R8) into each well in the same order and at the same speed of the substrate solution addition. Shake the microtiter plate gently to mix.

12.10 Read OD at 450nm within 5 min after addition of the stopping solution.

13. DATA ANALYSIS

The standard curve is displayed between concentration of mannan as X-axis (logarithmic scale) and optical density as Y-axis (linear scale). The standard curve is plotted by a logarithmic regression using X (Logarithmic) and Y (Linear). Determine the concentration of mannan in serum against the standard curve.

14. QUALITY CONTROL

14.1 Substrate Blank: the OD must be < 0.2;

14.2 Control A: The concentration must be 2.5μg/L-5.0μg/L;

14.3 Control B: The concentration must be < 0.5μg/L;

14.4 The r2 of the standard curve must be ≥ 0.98;

14.5 If these criteria are unmet, the test needs to be re-performed.

15. INTERPRETATION OF RESULTS

15.1 Concentration of mannan < 0.5μg/L indicates a negative result.

15.2 Concentration of mannan > 1.0μg/L indicates a positive result.

15.3 0.5μg/L ≤ Concentration of mannan ≤ 1.0μg/L indicates an inconclusive result. It is recommended to resample within a week.

Note:

(1) When the concentration of mannan is beyond the range of the standard curve:

OD sample > Standard R2a, it indicates a negative result.

OD sample < Standard R2e, it indicates a positive result. The sample is recommended being diluted and retested.

(2) If the square of correlation coefficient of the regression equation (r2) is lower than 0.98, it indicates the standard curve is unacceptable and a new test is needed.

16. CLINICAL PERFORMANCE

Mannan concentrations were determined in 53 patients with invasive Candidiasis，30 patients with bacterial infection，28 patients with skin Candida infection and 20 healthy subjects (healthy control group) by this assay. [2]

Sensitivity

66.1% using 1.0μg/L as cut-off

73.6% using 0.6μg/L as cut-off

Specificity

96.2% using 1.0μg/L as cut-off

87.1% using 0.6μg/L as cut-off

17. LIMITATIONS OF THE PROCEDUR

17.1 A negative test cannot rule out the diagnosis of invasive candidiasis because of the very low concentration and the rapid elimination of the mannan antigen during infection.

A diagnosis of invasive candidiasis can be made only if considering together the clinical, therapeutic, radiological, cytological, direct mycological and serological data, with any single criteria being interpreted with caution.

17.2 A negative test for mannan antigen must also be interpreted in conjunction with the results of anti-mannan antibody test: even in case of invasive candidiasis, the mannan antigen is more difficult to detect in patients tested positive for anti-mannan antibodies.

17.3 The performance of the detection of mannan antigen in serum is related to the frequency of the tests performed in the patient. Regular monitoring of high-risk patients and screening for anti-mannan antibodies are recommended in order to increase the sensitivity and early positivity of the test.

17.4 The Dynamiker Candida Mannan Assay procedure and the interpretation of the results must be followed when testing samples for the presence of mannan antigen .The user of the kit is advised to read the package insert carefully prior to conducting the test. In particular, the test procedure must be carefully followed for sample and reagent pipetting, plate washing, and timing of the incubation steps.

17.5 Failure to add specimen or reagents as instructed in the procedure could result in a falsely negative result. Repeat testing of additional samples should be considered where there is clinical suspicion of invasive candidiasis or procedural error.

17.6 Contamination of the negative patient specimen wells by positive control, calibrators or patient sample wells is possible if the contents of one well spill over into another well due to rough handling of the microplate or a poor pipetting technique while adding reagents.

17.7 The performance characteristics of Dynamiker Candida Mannan Assay have not been evaluated with neonatal or pediatric serum samples.

17.8 The Performance characteristics of Dynamiker Candida Mannan Assay have not been established for manual reading and/or visual result determination.

18. REFERENCE

[1] 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.

[2] JIANG Yueting，XIAO Yiwen，SU Danhong，YI Jianyun et al. The determination of mannan for the diagnosis of invasive candidiasis. Laboratory Medicine，：1673-8640（2016）07-0603-04.

19. MANUFACTURER

Company Name: Dynamiker Biotechnology (Tianjin) Co., Ltd.
Registration Address: No. 101-2, 14th Building, Ecological Science Park
No. 2018 Zhongtian Revenue, Eco-City TEDA, Tianjin 300467, P. R. China

[EC REP]

Company Name: Wellkang Ltd.
Address: Suite B, 29 Harley Street, LONDON W1G 9QR, England, United Kingdom

[SYMBOLS USED]

Symbol	Description
	Use By
LOT	Batch Code
	Manufacturer
	Keep Away from Sunlight
	Temperature Limitation
IVD	In Vitro Diagnostic Medical Device

EC REP	Authorized Representative in the European Community
CE	CE Mark

REVISED: 10/2017