Dynamiker Biotechnology (Tianjin) Co., Ltd.

Dynamiker *Aspergillus* Galactomannan Assay

Catalogue No.: DNK-1402-1

User Manual / 96 tests

CONTENTS

1. INTENDED USE

Dynamiker *Aspergillus* Galactomannan Assay is based on competitive Enzyme-linked Immunosorbent Assay (ELISA). It is used for the quantitative detection of *Aspergillus* glactomannan antigen in human serum and bronchoalveolar lavage fluid (BAL), offering a diagnostic reference for *Aspergillus* infection. The kit is intended for professional use only.

2. PRINCIPLE

Pipette the treated surem or BAL and the anti-galactomannan antibody respectively into wells coated with galactomannan antigen and then incubate. After removing the unbound material by washing, pipette the conjugate into wells and incubate. Again, after removing the unbound material by washing, the substrate solution is added and incubated. Then the stopping solution is added to terminate the color development. The result is measured at 450nm using an ELISA microplate reader. The intensity of color development is negatively proportional to the concentration of galactomannan antigen tested.

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, the invasive fungal diseases (IFD), with a high mortality, is increasing and complicated. The invasive *Aspergillosis* (IA) is rapidly increasing. The susceptible population is mainly people who receive immunosuppressive therapy, such as hematopoietic stem cell transplantation patients, hematic malignant carcinoma patients, solid organ transplantation patients, bone marrow transplantation patients as well as long-term chemotherapy and corticosteroid therapy patients and severe AIDS patients.

The clinical symptom of IA is non-specific. There are no identical features in CT scan and X-ray. The difficulty of early diagnosis and timely treatment results in a high mortality of 60%~100% [1]. The presence of galactomannan antigen against *Aspergillus* indicates a prior *Aspergillus* infection.

4. KIT COMPONENTS

No.	Component	Content	Quantity
R1	Microtiter Strips	12 breakable strips with 8 wells each; coated with *Aspergillus* galactomannan antigen	1 plate/ 12×8 wells
R2a	Standard a (0.25 μg/L)	Galactomannan antigen in PBS with protein; Preservative: 0.05% ProClin300	1×1mL
R2b	Standard b (0.5 μg/L)	Galactomannan antigen in PBS with protein; Preservative: 0.05% ProClin300	1×1mL
R2c	Standard c (1 μg/L)	Galactomannan antigen in PBS with protein; Preservative: 0.05% ProClin300	1×1mL

R2d	Standard d (2.5 μg/L)	Galactomannan antigen in PBS with protein; Preservative: 0.05% ProClin300	1×1mL
R2e	Standard e (5 μg/L)	Galactomannan antigen in PBS with protein; Preservative: 0.05% ProClin300	1×1mL
R3	Anti-Galactomannan Antibody	Anti-galactomannan antibodies, stabilized with protein stabilization solution	1×8mL
R4	Conjugate	Goat-anti-rabbit antibodies, conjugated with HRP; stabilized with protein stabilization solution	1×12mL
R5	Sample Treatment Solution	EDTA Solution	1×10mL
R6	Concentrated Washing Solution (20×)	PBS and Tween 20 Preservative: 0.05% ProClin300	1×12mL
R7	Sample Dilution Solution	PBS with protein and Tween 20 Preservative: 0.05% ProClin300	1×5mL
R8	Substrate Solution	Tetramethylbenzidine (TMB)	1×12mL
R9	Stopping Solution	2M H_2SO_4	1×8mL
R10	Control A	Galactomannan antigen in PBS with protein; Preservative: 0.05% ProClin300; >2.5μg/L	1×1mL
R11	Control B	Galactomannan antigen in PBS with protein; Preservative: 0.05% ProClin300; < 0.5μg/L	1×1mL
M1	Plate Sealer	Adhesive membrane of microtiter plate	1 sheet

5. STORAGE AND STABILITY

Item	Storage	Stability
Microtiter Strips coated with *Aspergillus* galactomannan antigen	after opening, stored in the sealed bag with desiccant at 2~8℃	4 weeks
Standards (a, b, c, d and e)	after opening, stored at 2~8℃	4 weeks
Controls (A and B)	after opening, stored at 2~8℃	4 weeks
Anti-Galactomannan Antibody	after opening, stored at 2~8℃	until expiry date
Conjugate	after opening, stored at 2~8℃	until expiry date
Sample Treatment Solution	after opening, stored at 2~8℃	until expiry date

Concentrated Washing Solution	after opening, concentrated solution (20×) stored at 2~8℃	until expiry date
	after dilution, washing solution stored at 2~8℃	2 weeks
Sample Dilution Solution	after opening, stored at 2~8℃	until expiry date
Substrate Solution	after opening, stored at 2~8℃ in dark	until expiry date
Stopping Solution	after opening, stored at 2~8℃ or room temperature	until expiry date

6. MATERIALS NEEDED BUT NOT SUPPLIED

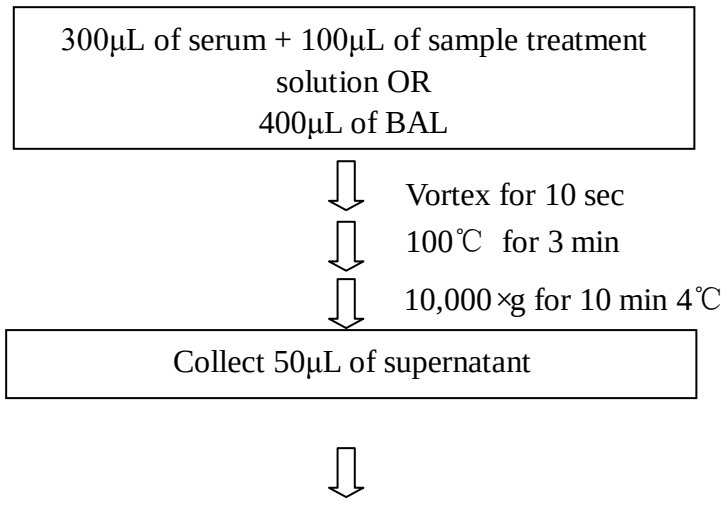
6.1 ddH_2O: for the dilution of concentrated washing solution
6.2 Absorbent paper
6.3 Disposable gloves
6.4 Pipette tips (200μL, 300μL, 1000μL)
6.5 Polypropylene centrifuge tubes (0.6mL or 1.5mL, sealed and gas-tight)
6.6 Vortex mixer
6.7 Water bath
6.8 Incubator
6.9 Semi-automatic plate washer (Recommended)
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 inside sealed tubes to avoid exposure to air in the process of transfer and storage. For longer storage, store the serum below -20℃. Avoid repeated freezing and thawing.
Serum samples can be stored at 2~8℃ for up to 48 hours before testing.

8. FLOW CHART OF TESTING PROCEDURE

Samples pretreatment:



Addition of sample/standards/controls:

Add 50μL supernatant/ standards/ controls + 50μL anti-galactomannan antibody into microtiter wells

⇩ Shake plate for 10 sec

⇩ 37℃ for 90 min

Wash:

Wash 3×300μL (1:20)

⇩

Addition of conjugate:

Conjugate: 100μL/ well except substrate blank

⇩ 37℃ for 30 min

Wash 3×300μL (1:20)

⇩

Addition of substrate solution:

TMB solution: 100μL/ well

⇩ 37℃ for 15 min+ light-proof

Termination of the reaction:

Stopping solution: 50μL/ well

⇩ In 5 min

Measurement:

Read OD at 450nm

9. SAMPLE TREATMENT

9.1 Treatment of Serum

9.1.1 Add 300μL of serum into the centrifuge tube.

9.1.2 Add 100μL of sample treatment solution (R5) into the above centrifuge tube.

9.1.3 Vortex the centrifuge tube for 10 sec. Heat the tube at 100℃ for 3 min in water bath.

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

9.1.5 Collect 50μL of supernatant for detection.

9.2 Treatment of BAL

9.2.1 Add 400μL of BAL into the centrifuge tube.

9.2.2 Vortex the centrifuge tube for 10 sec. Heat the tube at 100℃ for 3 min in water bath.

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

9.2.4 Collect 50μL of supernatant for detection.

10. PROCEDURE

10.1 Bring all reagents under room temperature (20-25℃) for 30 min before test.

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

10.3 Prepare washing solution:

Dilute the concentrated washing solution (20×) at 1:20 ratio with ddH_2O (e.g. 1mL conc. washing solution + 19mL ddH_2O). The resultant washing solution is stored at 2~8℃ for up to 2 weeks. Adequate washing solution should be prepared for the entire test.

10.4 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 *Aspergillus* galactomannan antigen separately, and then add 50μL of anti-galactomannan antibody into each well. Add 100μL of sample dilution into one well as the substrate blank.

Well	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	

10.5 Vortex the plate well for 10 sec. Seal the microtiter plate with a plate sealer and incubate it at 37℃ for 90 min.

10.6 Remove the plate sealer and shake out 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.

10.7 Add 100μL of conjugate into each well except the substrate blank.

10.8 Seal the microtiter plate with a plate sealer and incubate it at 37℃ for 30 min.

10.9 Repeat step 10.6.

10.10 Add 100μL of substrate solution into each well including the substrate blank.

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

10.12 Add 50μL 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.

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

11. DATA ANALYSIS

Subtract the OD value of the blank control from that of each well before analysis.

The standard curve is displayed between concentration of galactomannan as X-axis (logarithmic scale) and optical density as Y-axis (linear scale). The standard curve is estimated

by a logarithmic regression. Determine the concentration of galactomannan in serum or BAL against the standard curve.

12. QUALITY CONTROL

Substrate Blank: the OD must be < 0.2;
Control A: The concentration must be >2.5μg/L;
Control B: The concentration must be < 0.5μg/L;
If these criteria are unmet, the test needs to be re-performed.

13. INTERPRETATION OF RESULTS

13.1 Concentration of galactomannan < 0.65μg/L indicates a negative result.
13.2 Concentration of galactomannan ≥ 0.85μg/L indicates a positive result.
13.3 0.65μg/L ≤ Concentration of galactomannan < 0.85μg/L indicates an inconclusive result. It is recommended to resample within a week.

Note:

(1) When the concentration of galactomannan 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 (r^2) is lower than 0.98, it indicates the standard curve is unacceptable and a new test is needed.

14. PRECAUTIONS

14.1 Prevent samples and reagents from contamination of fungi and bacteria.
14.2 Use a separate micropipette or individual disposable tips to avoid carry-over and cross-contaminations.
14.3 Use reagents with the same lot.
14.4 Chemical reagents (acid or alkaline) or dust may affect the activity of conjugate.
14.5 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.
14.6 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.

15. WARNINGS

15.1 Don't pipette by mouth.
15.2 Don't smoke, eat or drink in areas where samples or kit reagents are handled.
15.3 Wear disposable gloves, laboratory coat and safety glasses when handling the kit reagents and patients samples. Wash hands thoroughly after testing.
15.4 All the used samples or consumptive materials must be treated as infectious medical wastes.

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

16. REFERENCE

[1] Update on invasive aspergillosis: clinical and diagnostic aspects. P. Munoz, J. Guinea and E. Bouza, *Clin Microbiol Infect* 2006, 12 (7): 24–39

17. MANUFACTURER

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[EC REP]

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[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

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