Dynamiker Biotechnology (Tianjin) Co., Ltd.

Dynamiker *Aspergillus fumigatus* IgG Assay

Catalogue No.: DNK-1407-1

User Manual / 96 tests

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1. INTENDED USE

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

2. PRINCIPLE

The diluted serum samples are pipetted into the wells coated with galactomannan antigen and then incubated. 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 incubated to form immune complexes. The substrate solution is pipetted and incubated after further washing. 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 proportional to the concentration of IgG-specific antibodies detected.

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 Disease, with a high mortality, is increasing and complicated. The Invasive *Aspergillosis* (IA) is rapidly increasing and *Aspergillus fumigatus* is the major pathogenic fungus which occupies 80%~90% of IA [1]. 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, 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. The presence of IgG antibodies against *Aspergillus fumigatus* indicates a prior *Aspergillus fumigatus* 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 *Aspergillus fumigatus* galactomannan antigen	1 plate / 12×8 wells
R2a	Standard a (500 AU/mL)	*Aspergillus fumigatus* IgG antibodies in PBS with protein; Preservative: 0.05% ProClin300	1×1mL

R2b	Standard b (250 AU/mL)	*Aspergillus fumigatus* IgG antibodies in PBS with protein; Preservative: 0.05% ProClin300	1×1mL
R2c	Standard c (125 AU/mL)	*Aspergillus fumigatus* IgG antibodies in PBS with protein; Preservative: 0.05% ProClin300	1×1mL
R2d	Standard d (62.5 AU/mL)	*Aspergillus fumigatus* IgG antibodies in PBS with protein; Preservative: 0.05% ProClin300	1×1mL
R2e	Standard e (31.25 AU/mL)	*Aspergillus fumigatus* 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_2SO_4	1×8mL
R8	Control A	*Aspergillus fumigatus* IgG antibodies dissolved in PBS with protein; Preservative: 0.05% ProClin300 Concentration: 125-250 AU/mL	1×1mL
R9	Control B	*Aspergillus fumigatus* 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

Item	Storage	Stability
Microtiter Strips coated with *Aspergillus fumigatus* 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
Conjugate	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 2-30℃	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 2~30℃	until expiry date
Controls (A and B)	after opening, stored at 2~8℃	4 weeks

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 (10μL, 50μL, 100μL, 1,000μL)
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 inside sealed tubes to avoid exposure to air in the process of transfer and storage. For longer storage, store the serum or plasma below -20℃. Avoid repeated freezing and thawing. Serum or plasma samples can be stored at 2-8℃ for up to 5 days before testing.

8. FLOW CHART OF TESTING PROCEDURE

Samples pretreatment:

Pipette 1μL of samples into 1,000μL of sample dilution and mix thoroughly.

⇩

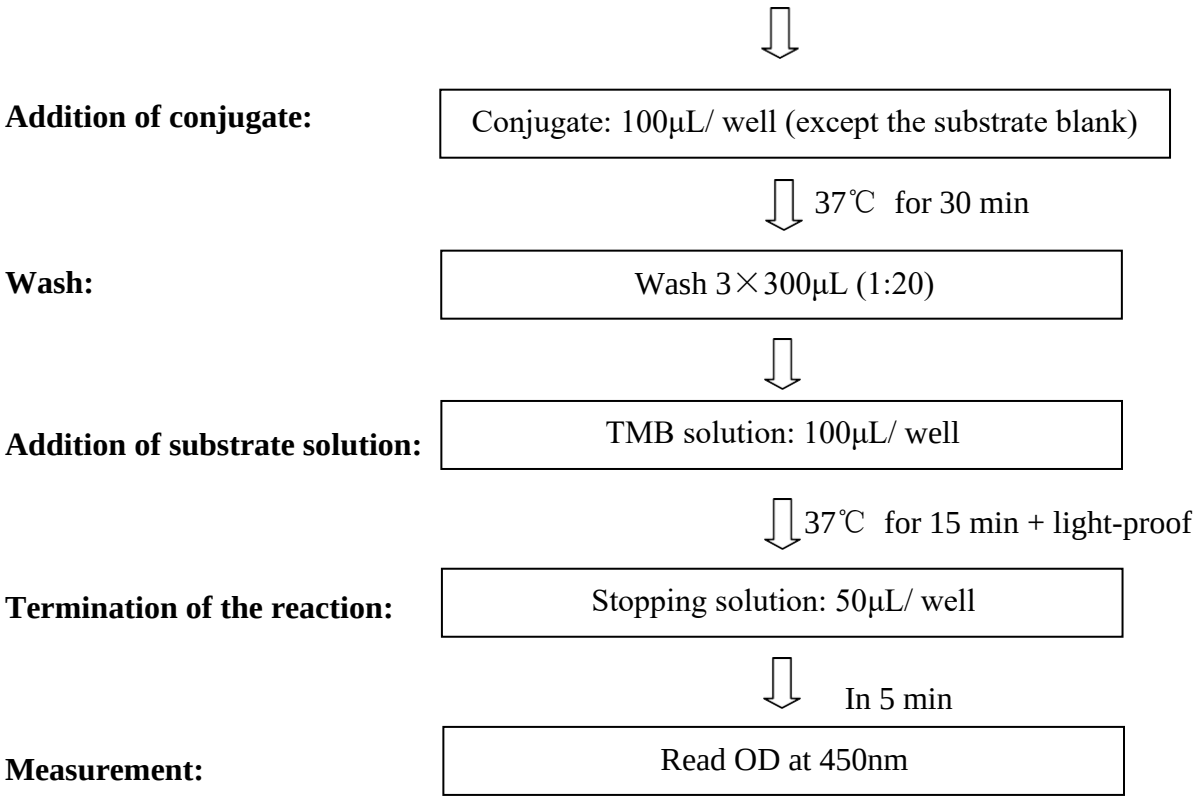
Addition of standards /controls /samples:

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

⇩ 37℃ for 60 min

Wash:

Wash 3×300μL (1:20)



9. PROCEDURE

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

9.2 Take the microtiter strips out of the sealed bag (R1). Return the unused strips and seal the bag tightly.

9.3 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.

9.4 Sample dilution solution:
Pipette 1μL of sample into 1,000μL of sample dilution. Vortex the mixture well.

9.5 Leave one well for a substrate blank.

9.6 Pipette 100μL of the standards, controls and diluted serum 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 it for 60 min at 37℃.
9.8 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 the last wash, invert the microtiter plate and dry it by tapping on the absorbent paper.
9.9 Add 100μL of conjugate into each well except the substrate blank.
9.10 Seal the microtiter plate with a plate sealer and incubate it at 37℃for 30 min.
9.11 Repeat **step 9.8**.
9.12 Add 100μL of substrate solution into each well including the substrate blank.
9.13 Incubate the microtiter plate at 37℃ for 15 min without sealing.
9.14 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.
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-galactomannan 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-galactomannan 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-galactomannan antibody < 80 AU/mL indicates a negative result.
2. Concentration of IgG anti-galactomannan antibody ≥ 120 AU/mL indicates a positive result.
3. 80 AU/mL ≤ Concentration of IgG anti-galactomannan antibody < 120 AU/mL indicates an inconclusive result. It is recommended to resample within a week.

Note:

1. When the concentration IgG anti-galactomannan 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 while 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 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] Epidemiology and Prevention of Invasive Aspergillosis. David W. Warnock, et al. *Current Infectious Disease Reports*, Volume 3, Number 6, 507-516

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17. MANUFACTURER

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[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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