Dynamiker Biotechnology (Tianjin) Co., Ltd.

Dynamiker *Aspergillus* Galactomannan Assay

Catalogue No.: DNK-1402-1

User Manual / 96 tests

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1. INTENDED USE

Dynamiker Aspergillus Galactomannan Assay is based on sandwich Enzyme-linked Immunosorbent Assay (ELISA). It is used for the detection of *Aspergillus* galactomannan 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 serum or BAL into wells coated with galactomannan antibody and then incubate. After removing the unbound material by washing, pipette the conjugate into wells and incubate. An antibody - galactomannan - antibody / peroxidase complex is formed in the presence of galactomannan antigen. Again, 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 absorbance (optical density) of specimens and controls is determined with a spectrophotometer set at 450 and 620/630 nm wavelength.

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 of 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 *anti*-galactomannan antibodies	1 plate/12×8 wells
R2	Negative control serum	Negative Control Serum: - Freeze-dried human serum negative for galactomannan - Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs antigen	2×1.7mL
R3	Cut-off control serum	Cut-off Control Serum: - Freeze-dried human serum containing galactomannan - Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs antigen	2×1.7mL

R4	Positive control serum	Positive Control Serum: - Freeze-dried human serum containing galactomannan - Negative for anti-HIV-1, anti-HIV-2, anti-HCV antibodies and HBs antigen	2×1.7mL
R5	Conjugate	Anti-galactomannan antibodies, conjugated with HRP; Preservative: 0.01% thimerosal	1×12mL
R6	Sample Treatment Solution	EDTA Solution	1×12mL
R7	Concentrated Washing Solution (20×)	PBS and Tween 20, PH 7.0-7.4 Preservative: 0.05% ProClin300	1×50mL
R8	Substrate Solution	Tetramethylbenzidine (TMB)	1×12mL
R9	Stopping Solution	2M H_2SO_4	1×8mL
M1	Plate Sealer	Adhesive membrane of microtiter plate	3 sheet

5. STORAGE AND STABILITY

5.1 The kit can be stored at 2-8 ℃ for up to 12 months after production.

5.2 After opening or dilution, please refer to the storage condition and time as below.

Item	Storage	Stability
Microtiter Strips coated with *anti-galactomannan* antibodies	After opening, stored in the sealed bag with desiccant at 2~8℃	8 weeks
Controls (Negative/Cut-off/Positive)	After opening, stored at 2~8℃	8 weeks
Conjugate	After opening, stored at 2~8℃	8 weeks
Sample Treatment Solution	After opening, stored at 2~8℃	8 weeks
Concentrated Washing Solution	After opening, concentrated solution (20×) stored at 2~8℃	8 weeks
	After dilution, washing solution stored at 2~30℃	2 weeks
Substrate Solution	After opening, stored at 2~8℃ in dark	8 weeks
Stopping Solution	After opening, stored at 2~30℃	8 weeks

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 and BAL fluid 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 OR BAL FLUID 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 (R7) 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 Use clean, dust-free materials (tubes, tips, containers, etc.) to minimize the possibility of contamination with Aspergillus spores from the environment. Because galactomannan is heat-stable, sterilization of material used does not guarantee the absence of contaminating antigen. Pyrogen-free materials are optimal, but standard material can be used with adequate precautions.
7.16 Limit exposure of solutions (sera, BAL fluid, Sample Treatment Solution, Conjugate) or open containers (plates, tubes, pipettes) to the air.
7.17 Do not pour any unused Conjugate back into the original container.
7.18 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 ddH_2O: for the dilution of concentrated washing solution
8.2 Absorbent paper
8.3 Disposable gloves
8.4 Pipette tips (200µL, 300µL, 1000µL)
8.5 Pipette (100uL, 1000uL)
8.6 Centrifuge (**10,000 x g**)
8.7 Polypropylene centrifuge tubes (0.6mL or 1.5mL, sealed and gas-tight)
8.8 Vortex mixer
8.9 Water bath or Heat block
8.10 Incubator
8.11 Semi-automatic plate washer (Recommended)
8.12 Microplate reader with 450 nm and 620/630 nm

9. SAMPLE COLLECTION AND STORAGE

This test is performed on serum or BAL fluid.

9.1 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 72 hours prior to testing. For longer storage, store the serum at -20°C or lower.
Avoid repeated freezing and thawing.

9.2 BAL FLUID

Collect BAL fluid samples according to standard laboratory procedures. The recommended lavage volume is 100mL (20mL each time and repeat 5 times), and the recovery rate is 40% to 60%. For some location with low lavage volume, the recovery rate should no less than 30%. BAL fluid samples must be collected in sterile saline and may be tested on neat samples (as is) or supernatants from centrifuged samples (10,000 rpm for 10 min).
BAL fluid 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 up to 72 hours. For longer storage, store the BAL samples at -20°C or less.
Avoid repeated freezing and thawing.

10. FLOW CHART OF TESTING PROCEDURE

Samples pretreatment:

300μL of serum/BAL/Negative control/Cut-off control/Positive control + 100μL of sample treatment solution

⇩ Vortex for 10 secs

⇩ 10,000×g for 5 seconds

⇩ 100℃ for 3 mins

⇩ 10,000×g for 10 mins 4℃

Collect 100μL of supernatant

⇩

Addition of sample/controls:

Add 100μL supernatant into microtiter wells (Cut-off control in duplicate)

⇩ 37℃ for 60±5 mins

Wash:

Wash 1×300μL (1:20)

⇩

Addition of conjugate:

Conjugate: 100μL/ well

⇩ 37℃ for 30±3 mins

Wash 3×300μL (1:20)

⇩

Addition of substrate solution:

TMB solution: 100μL/ well

⇩ 37℃ for 25±2 mins + light-proof

Termination of the reaction:

Stopping solution: 50μL/ well

⇩ In 5 mins

Measurement:

Read OD at 450nm

11. SAMPLE TREATMENT

11.1 Treatment of Serum and BAL

All control sera: Negative control serum (R2), Cut-off control serum (R3) and Positive control serum (R4) must be processed at the same time as serum/BAL fluid samples.

1. Pipette 300μL of serum/BAL and control sera into centrifuge tubes. Cut-off control serum (R3) use two tubes.
2. Add 100μL of sample treatment solution (R6) into each tube.
3. Vortex the centrifuge tube for 10 sec. **Centrifuge the tubes at 10,000× g for 5 seconds**. Heat the tube at 100℃ for 3 mins in water bath. Tightly close the tube to prevent opening during heating.

NOTE: Centrifuge the tubes after vortex is important to make the liquid on the caps fall down into the tubes.

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.

5. Heat block option:

Heat tubes for **6 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 turned on at least 20 mins 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.

6. Centrifuge the heated tube for 10 mins at 10,000×g (at 4 ℃ if centrifuge is refrigerated). If the centrifuge does not have 4 ℃ function, then place the tubes at room temperature for 10mins before added into the microplate.

Note: If the customer hope to do duplicate of samples, then prolong the centrifuge time to 20mins.

7. Collect 100μL of supernatant for detection.
8. Test the supernatants using the following procedure. After preparation, the supernatant may be collected/saved and stored at 2-8°C for up to 48 hours prior to testing. If analysis of the results indicates that retesting is required, another aliquot of the sample must be treated for testing.

12. ELISA PROCEDURE

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

12.2 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 diluted washing solution is stored at 2~30℃ for up to 2 weeks. Adequate washing solution should be prepared for the entire test.

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

12.4 Prepare a chart for identification of test sera/BAL and controls in the microplate. Use one well for the Negative Control Serum (R2), two wells for the Cut-off Control Serum (R3), and one well for the Positive Control Serum (R4).

	1	2	3	4	5	6	7	8	9	10	11
A	Negative control(R2)	Sample 5	Sample 13								
B	Cut-off control(R3)	Sample 6	……								
C	Cut-off control(R3)	Sample 7									
D	Positive control(R4)	Sample 8									
E	Sample 1	Sample 9									
F	Sample 2	Sample 10									
G	Sample 3	Sample 11									
H	Sample 4	Sample 12									

12.5 Pipette 100uL of supernatant of treated serum/BAL, Negative control, Cut-off control (duplicate) and positive control to each well as designed by above.
12.6 Cover the microtiter plate with a plate sealer and incubate it at 37℃ for 60±5 mins.
12.7 Remove the plate sealer and aspirate the incubation solution. Wash the wells **ONCE** with 300μL/ well washing solution. **The soak time is 40 secs**. After wash, invert the microtiter plate and dry it by tapping on the absorbent paper.
12.8 Add 100μL of conjugate (R5) into each well.
12.9 Cover the microtiter plate with a plate sealer and incubate it at 37℃ for 30±3 mins.
12.10 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 secs**. After each wash, invert the microtiter plate and dry it by tapping on the absorbent paper.
12.11 Add 100μL of substrate solution (R8) into each well.
12.12 Incubate the microtiter plate at 37℃ and light-proof for 25±2 mins without sealing.
12.13 Add 50μL of stopping solution (R9) into each well in the same order and at the same speed of the substrate solution addition. Shake the microtiter plate gently to mix.
12.14 Read OD at 450nm (Reference 620/630nm) within 5 mins after addition of the stopping solution.

13. DATA ANALYSIS

The presence of galactomannan in the sample is determined by the Index value (I value) of each well. I value means: the OD of each wells divided by the Mean OD value of Cut-off control.

$$I = \frac{\text{OD of Samples}}{\text{Mean Cut-off Control OD}}$$

Example Calculation:

Sample	Absorbance (OD)
Negative control R2	0.15
Cut-off control R3	0.61
	0.63
Positive control R4	2.56
Sample 1	0.22
Sample 2	1.85

Calculation

Mean Cut-off control values

To calculate the mean Cut-off Control (R3) OD, add the OD values for each Cut-off Control replicate together and divide the result by 2:

$$\frac{(0.61+0.63)}{2} = 0.62$$

Negative control Index

To calculate the index of the Negative Control, divide the OD of the Negative Control by the mean Cut-off Control OD:

$$I = \frac{0.15}{0.62} = 0.24$$

Positive control Index

$$I = \frac{2.56}{0.62} = 4.13$$

Sample 1 Index

$$I = \frac{0.22}{0.62} = 0.35$$

Sample 2 Index

$$I = \frac{1.85}{0.62} = 2.98$$

14. QUALITY CONTROL

14.1 Negative control Index must be < 0.4;

14.2 Positive control Index must be >1.5;

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

15. INTERPRETATION OF RESULTS

The following cut off limits were identified in the population studied to obtain the performance characteristics, however each laboratory may wish to establish their own cut offs values and negative and positive interpretation with their patient population.

15.1 Serum Index <0.5 or BAL Index <1.0

Serum Index <0.5 or BAL Index <1.0 are considered to be Negative for galactomannan antigen.

Note: A negative result may indicate that the patient's result is below the detectable level of the assay. Negative results do not rule out the diagnosis of Invasive Aspergillosis. Repeat testing is recommended if the result is negative, but the disease is suspected.

15.2 Serum Index ≥0.5 or BAL Index ≥1.0

Serum Index ≥0.5 or BAL Index ≥1.0 are considered to be Positive for galactomannan antigen.

For all positive patients, it is recommended that a new aliquot of the same sample (serum/BAL) be repeated.

Note: An absorbance value of less than 0.000 may indicate a procedural or instrument error which should be evaluated. That result is invalid and the specimen must be re-run. Regular screening (twice-weekly) of serum samples of high-risk patients is recommended to increase the sensitivity and early positivity of the test.

Note: The Dynamiker Aspergillus Galactomannan assay is intended to be used as an aid in the

diagnosis of Invasive Aspergillosis. Positive results obtained with this assay should be considered in conjunction with other diagnostic procedures such as microbiological culture, histological examination of biopsy samples and radiographic evidence.

16. CLINICAL PERFORMANCE

A total of 82 hematological patients at risk of invasive Aspergillosis were tested by this assay including one proven patient, 28 probable patients, 23 possible and 30 patients with no IFD as control. [2]

Sensitivity: 79.3%

Specificity: 83.0%

17. LIMITATIONS OF THE PROCEDURE

17.1 A negative test from serum and/or BAL samples cannot rule out the diagnosis of Invasive Aspergillosis. Serum samples from patients at risk for Invasive Aspergillosis should be tested twice a week.

17.2 The Procedure and the Interpretation of Results must be followed when testing samples for the presence of galactomannan 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.

17.3 Failure to add specimen or reagent as instructed in the procedure could result in a falsely negative test. Repeat testing of additional samples should be considered where there is clinical suspicion of Invasive Aspergillosis or procedural error.

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

17.5 The concomitant use of mold-active anti-fungal therapy in some patients with Invasive Aspergillosis may result in reduced sensitivity with Dynamiker Aspergillus Galactomannan Assay.

17.6 The Dynamiker Aspergillus Galactomannan Assay has not been evaluated for use with plasma or other sample types such as urine or CSF.

17.7 The performance of the Dynamiker Aspergillus Galactomannan Assay has not been established for manual reading and/ or visual result determination.

17.8 Cross-reactivity of BAL fluid samples with Mycoplasma pneumoniae or anaesthetic drugs/lubricants used to numb the neck/throat area for the aspiration process has not been evaluated.

17.9 Positive reactions with no clinical signs

The following should be considered with regard to the early galactomannan antigen detection in serum or BAL before the appearance of clinical and/or radiological signs. Positive test results without clinical signs are usually observed and they have been shown to correspond to «true positive» tests in patients for whom Proven or Probable Invasive Aspergillosis diagnosis is established later on.

However, in some particular cases, specific factors should be taken into account when interpreting the test:

1) Positive test results with no clinical signs have been reported, especially in young children. Although some of these cases could be related to real circulation of Aspergillus antigens, most cases can be considered to be false-positives.

2) Galactofuranose has been demonstrated in various foods, particularly cereals, cereal products and cream desserts. Unlike human milk, cow's milk formulas frequently contain high

concentrations of galactomannan. Dietary factors must therefore be taken into account in interpretation of the course of antigenemia in young children, and more generally in all patients with an altered intestinal barrier. Any case of positive antigenemia not accompanied by clinical signs should be interpreted even more cautiously in this population of patients.

3) There have been reports of positive galactomannan test results in patients receiving piperacillin/ tazobactam. There have also been reports of certain lots or batches of piperacillin/ tazobactam that have been found to be positive for galactomannan antigen. Therefore, positive test results in patients receiving piperacillin / tazobactam should be interpreted cautiously and confirmed by other diagnostic methods. Detection of galactomannan has also been reported in some batches of amoxicillin associated with clavulanic acid parenteral preparations. Therefore, semi-synthetic ß-lactam treatments should be taken into account when interpreting the test. Nevertheless, as Aspergillus Galactomannan Assay can detect galactomannan antigen well before clinical or radiological signs appear, the occurrence of Invasive Aspergillosis cannot be ruled out. Therefore, patients treated with piperacillin/tazobactam with positive test results should be followed carefully.

4) Positive reactions in the absence of clinical signs may be observed in patients receiving products containing galactomannan, either parenterally or orally (in the presence of an alteration of the intestinal barrier). The presence of galactomannan in these products can often be explained by the use of a fermentation process based on fungal microorganisms. A positive result will not be observed in a patient, however, unless the serum concentration of exogenous galactomannan reaches or exceeds the test's detection threshold.

Thus, if there is a suspicious positive result in the absence of other clinical signs, we recommend investigating the products that the patient is taking and notably their production processes and the origin of the raw materials used.

18. 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

[2] XIAO Chenlu, Han Lizhong, Ni Yuxing, Guo Xiaokui et al: Correlation between dynamic monitoring of serum galactomannan antigen and antifungal treatment in adult hematologic patients at risk of invasive aspergillosis. Chin J Infect Chemother, 1009-7708(2015)04-0364-04.

19. MANUFACTURER

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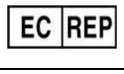
CE

[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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