Dynamiker Biotechnology (Tianjin) Co., Ltd.

Dynamiker Fungus (1-3)-β-D-Glucan Assay

Catalogue No.: DNK-1401-1

User Manual / 96 tests

CONTENTS

1. INTENDED USE

(1-3)-β-D-Glucan is the main cell wall component of most fungi, such as *Candida*, *Aspergillus* and *Fusarium*, etc. and does not exist in bacteria, virus or human cells.
Fungus (1-3)-β-D-Glucan Assay is based on spectrophotometry for the quantitative detection of (1-3)-β-D-Glucan in human serum. It offers a diagnostic reference for invasive fungal diseases. The kit is intended for professional use only.

2. PRINCIPLE

The Dynamiker Fungus (1-3)-β-D-Glucan Assay is based on pathways as shown below (Figure 1). The pre-treated sera are added into the Main Reagent which contains Factor G. Factor G is activated by (1-3)-β-D-Glucan and activated Factor G converts proclotting enzyme to clotting enzyme. The clotting enzyme hydrolyzes the substrate (Boc-leu-Gly-Arg-PNA) to release PNA. The absorbance is measured at 405nm kinetically. The concentration of (1-3)-β-D-Glucan is interpreted according to a standard curve.

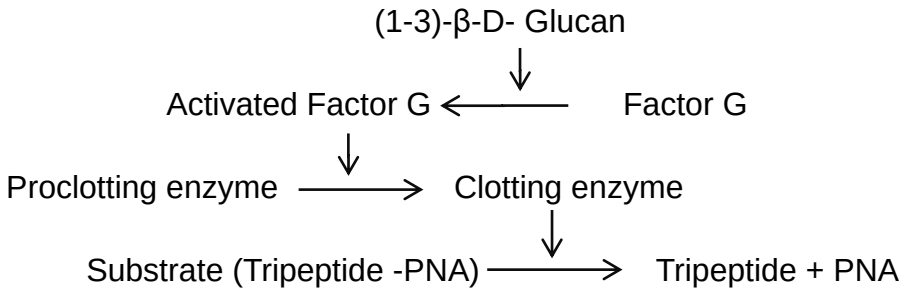

Figure 1

3. SUMMARY AND EXPLANATION

In recent years, with the rising numbers of stem cell transplantation and solid organ transplant recipients; increasing use of excessively high dose immunosuppressant and aggressive chemotherapy; widespread use of interventional therapy and indwelling of catheter, the incidence of invasive fungal diseases (IFD) have increased significantly. IFD becomes a major cause of death and severe complications for patients who receive bone marrow or organ transplant and patients who receive chemotherapy due to malignant hematopathy and tumor, AIDS patients and those in critical conditions. Diagnosis of invasive mycoses usually involves non-specific diagnostic or radiological techniques.

4. KIT COMPONENTS

No.	Component	Content	Quantity
R1	Main Reagent	G Factor and Proclotting enzyme	4×2.6mL
R2	Treatment Solution A	KOH Solution	1×3mL
R3	Treatment Solution B	KCl Solution	1×3mL

R4	Standard	(1-3)- β-D-Glucan lyophilized powder	5×1.5mL
R5	Control	(1-3)- β-D-Glucan lyophilized serum	5×1.5mL
R6	Diluent	Deionized Water	3×8mL
R7	Reconstitution Solution	Tris-HCl Buffer	4×3mL
R8	Breakable Microplate	-	12×8 wells

5. MATERIALS NEEDED BUT NOT SUPPLIED

All disposable materials must be glucan-free.

5.1 Disposable gloves (powder free)
5.2 Sterile vacuum tubes
5.3 Centrifuge
5.4 Pipette (Adjustable, 5-25μL and 200-1000μL)
5.5 Vortex mixer
5.6 Timer
5.7 Transfer tubes (for dilution of sample or standard)
5.8 Pipette tips (200μL and 1000μL)
5.9 Photometer with kinetic reading and 37℃ incubation function

6. STORAGE AND STABILITY

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

7. SAMPLE COLLECTION AND STORAGE

7.1 Collect 4mL of blood samples according to the standard laboratory procedures using glucan free sterile vacuum tubes. Make sure the sample is not contaminated by fungal spores or bacteria.
7.2 Centrifuge the blood sample at room temperature at 400xg for 10 to 15 min.
7.3 Collect serum and carry out the test in less than 2 hours.
7.4 The serum should be stored at 2-8℃ for not more than 24 hours. Avoid repeated reconstitution of the serum sample. It is possible to freeze serum sample once only.
7.5 There may be inaccurate results for some special samples: Hemolysis, turbid sample with high lipid concentration, jaundice.

8. PROCEDURE

Place the kit at room temperature for 30 minutes before testing.

The test is recommended to be performed on a clean bench to avoid contamination.

8.1 Preparation of Standard Solution

8.1.1 Dissolve one vial of Standard (R4) with 1.5mL of Diluent (R6) to make a Solution A (200pg/mL). Vortex for at least 1 min.

8.1.2 Make serial dilution from Solution A to prepare solutions B, C, D and E step by step.

Serial dilution	Prepared Solution
(1-3)-β-D-Glucan Standard	200pg/mL Solution A
Solution A 0.5mL + Diluent 0.5mL	100pg/mL Solution B
Solution B 0.5mL + Diluent 0.5mL	50pg/mL Solution C
Solution C 0.5mL + Diluent 0.5mL	25pg/mL Solution D
Solution D 0.5mL + Diluent 0.5mL	12.5pg/mL Solution E

Note:

(1) All the above solutions are prepared for the standard curve. The standard concentration should be multiplied by three, so that the standard concentration for solutions A, B, C, D and E should be set as 600pg/mL, 300pg/mL, 150pg/mL, 75pg/mL and 37.5pg/mL.

(2) As in total 5 individual vials of standard and control are provided for each kit, freezing used standards and controls are not recommended. Fresh standards and controls should be used for each run.

8.2 Reconstitution of positive control

Add1.5mL of Diluent (R6) to the bottle containing the Control (R5). Vortex for at least 1 min.

Note:

If the control needs to be used repeatedly, split the package appropriately and store under -20℃ for not more than 30 days. The control can only be frozen and thawed once. Thaw the control before usage and vortex for 30 sec to mix thoroughly.

8.3 Addition of negative control

Add 60μL of Diluent (R6) into one well as negative control.

8.4 Addition of Standard Solutions

Add 60μL of Standard Solutions (A, B, C, D and E) respectively into microplate wells.

8.5 Preparation of Treatment Solution

Mix Treatment Solution A (R2) and B (R3) at the ratio of 1:1 to make a Pre-treatment Solution.

8.6 Addition of Serum and Pre-treatment Solutions

8.6.1 Add 20μL of serum (or control) into microplate wells.

8.6.2 Transfer 40μL of Pre-treatment Solutions into the microplate wells that contain 20uL of serum sample (or control) added in Step 8.6.1.

8.6.3 Shake for 5-10 seconds and incubate the microplate at 37℃ for 10 min.

8.7 Addition of Main Reagent

8.7.1 Resolve one vial of Main Reagent (R1) by first adding 1.3mL of Reconstitution Solution

(R7) and then 1.3mL of Diluent (R6). Mix thoroughly and ready to use. It is recommended to resolve the Main Reagent during incubation of samples.

8.7.2 Add 100μL of resolved Main Reagent into the microplate wells.

8.7.3 Shake the plate for 5-10 sec.

8.7.4 Read OD value at 37 ℃ kinetically at 405nm and 490nm for 40 min.

9. DATA ANALYSIS

Take mAbs/min as the Y-axis and the Concentration of 1-3 β-D-Glucan as X-axis, plot the standard curve by linear regression. Determine the concentration of 1-3 β-D-Glucan of the samples and control against this standard curve.

10. QUALITY CONTROL

10.1 The critical time of a negative control (mAbs/min) must be longer than that of the minimum concentration. That indicates the test operation is free of contamination.

10.2 If there is a large deviation in the standard curve, it is recommended to repeat the test.

10.3 The square of correlation coefficient r2 must be > 0.980.

10.4 The calculated range of Quality Control must fall within the range as shown on the label of Quality Control bottle.

11. 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.

Result < 70pg/mL indicates a negative result.

Result > 95pg/mL indicates a positive result.

70pg/mL < Result < 95pg/mL indicates an inconclusive result. An inconclusive result indicates a suspected invasive fungal infection, additional sampling and assay is suggested.

Note:

The test does not detect Cryptococcus, Zygomycetes (such as Absidia, Mucor and Rhizopus) and yeast phase of Blastomyces dermatitidis.

12. CLINICAL PERFORMANCE

12.1 There were 163 serum samples from 121 patients tested in UK by this assay. Serum samples from patients with no evidence of fungal disease not attaining an EORTC/MSG diagnosis were included as controls. [3]

Sensitivity

The overall sensitivity for proven and probable samples by this assay is 81.4%(35/43, 95% CI: 67.4–90.3)

The sensitivity for invasive Candidiasis is 93.3% (14/15, 95% CI: 70.2–98.8).

The sensitivity for invasive Aspergillosis is 81.0% (17/21, 95% CI: 60.0-92.3).

Specificity

The overall specificity is 78.1%(50/64, 95% CI: 66.6–86.5)

12.2 There were 72 serum samples were tested in Peking Union Medical College, China by this assay. The sensitivity and specificity were 82.9% and 94.6%, respectively. [4]

12.3 77 serum samples from newborn infants with high risk of invasive fungal infection were classified based on blood culture into three groups: no fungemia (41 neonates with proven bacterial sepsis), suspected fungemia (25 neonates with negative blood culture), and definite fungemiagroup (11 neonates with culture-proven Candida). All the samples tested by this assay. [5]

Sensitivity: 63.6%

Specificity: 95.1%

12.4 A total of 38 serum samples were tested by Dynamiker 1-3 Beta-D Glucan assay, of which 16 with Invasive Aspergillosis, 7 with Invasive Candidosis, 5 with Pneumocystis Pneumonia and 10 samples from non-mycosis patients as control group. [6]

Sensitivity:

In Aspergillosis: 62.5%

In Candidiosis: 85.7%

In PJP: 80%

Specificity: 90%

13. LIMITATIONS

13.1 The (1-3)-β-D-Glucan test results is only used as a clinical reference in the diagnosis of deep-seated mycoses and fungemia, but cannot distinguish which fungal species may have caused the infection.

13.2 The sampling frequency is determined by the degree of infection. Patients at risk for IFD should be tested twice a week.

13.3 False positive results are caused by the following factors:

a. Contamination during the test;
b. Subjects that have hemodialysis with cellulose membranes;
c. Subjects that use glucan-containing gauze or related materials;
d. Intravenous preparations (albumin, blood coagulation factor, immunoglobulin, etc.);
e. Subjects presented with bacteria septicemia (streptosepticemia in particular);
f. Subjects who receive treatment with some antitumor drugs (lentinan and schizophyllan);
g. Subjects who receive treatment with sulfonamides;

14. WARNINGS

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 number.

14.4 Chemical reagents (acid or alkaline) or dusts may influence the activity of reagents.

14.5 Don’t pipette by mouth.

14.6 Don’t smoke, eat or drink in areas where samples or reagents are handled.

14.7 Wear disposable gloves, laboratory coat and safety glasses when handling the kit

reagents and patients samples. Wash hands thoroughly after test.

14.8 All the used samples or test materials must be treated as infectious medical wastes.

14.9 The components of the kit could lead to irritation and pain of the skin and eyes, and also can stimulate mucosa and upper respiratory tract. Do not touch, inhaler eat.

14.10 Special specimens such as jaundice, hematolysis, chyle will affect the assay results. If the degree of off-color or turbid is low, the specimens should be diluted before test. If such degree is high, then resampling is necessary.

15. REFERENCES

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[2] Kakinuma, A, Asano, T, et al: BiochemBiophys. Res. Commun.101,434-439 (1981).

[3] White PL, Price JS, Posso RB, Barnes RA, et al: An evaluation of the performance of the Dynamiker® Fungus (1-3)-β-D-Glucan Assay to assist in the diagnosis of invasive aspergillosis, invasive candidiasis and Pneumocystis pneumonia. Med Mycol. 2017 Nov 1;55(8):843-850. doi: 10.1093/mmy/myx004.

[4] Wang Y. Performance of the Dynamiker (1-3)-β-D-glucan assay compared to Fungitell for the diagnosis of invasive fungal disease from serum samples. Abstract no 5872; 26th European Congress of Clinical Microbiology and Infectious Diseases, 2016, Amsterdam, The Netherlands.

[5] K. Nikolai. Clinical evaluation of Dynamiker Aspergillus Galactomannan assay and Dynamiker 1-3 Beta-D Glucan assay.

16. MANUFACTURER

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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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