Dynamiker Biotechnology (Tianjin) Co., Ltd.

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

Catalogue No.: DNK-1401-1

User Manual / 96 tests

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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.

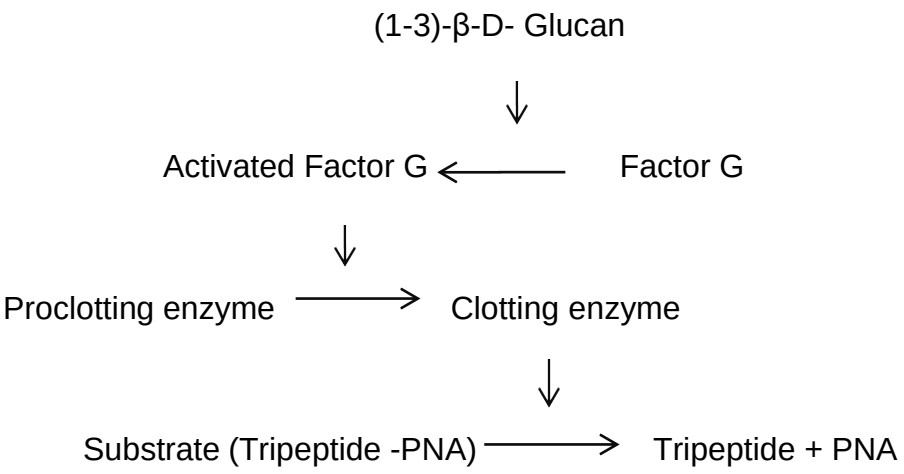

Figure 1

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

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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
R3	Treatment Solution	Alkaline Solution	4×1.5mL
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	4×8mL
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 blood collection tubes

5.3 Centrifuge

5.4 Pipette (Adjustable, 10-100μL/20-200μL and 100-1000μL)

5.5 Vortex mixer

5.6 Timer

5.7 Pyrogen-free transfer tubes (for dilution of sample or standard)

5.8 Pyrogen-free pipette tips (200μL and 1000μL), filter tips are recommended

5.9 Photometer with **kinetic** reading (405nm and 490nm) and **37℃ incubation function**

6. STORAGE AND STABILITY

6.1 The kit can be stored for up to 24 months at 2-8℃.

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6.2 See the expiry date from the label on the package. Don't use kit components from different lot kits in one assay.

6.3 After opening or reconstitution, please refer to the storage condition and time as below.

NO.	Components	After opening/reconstitution	Storage time
R1	Main reagent	Sealed with parafilm and stored at -20 ℃ or lower. Check the refrigerator in the lab	5 days
R3	Treatment solution	2-8 ℃	Until expiry date
R4	Standard	Discarded	Discarded
R5	Control	Discarded	Discarded
R6	Diluent	2-8 ℃	Until expiry date
R7	Reconstitution Solution	Discarded	Discarded
R8	Breakable Microplate	Seal unused strips and stored at 2-8 ℃	Until expiry date

Notes: Fresh Standards (R4) and Controls (R5) have to be used for each run.

If the color of the frozen opened Main reagent changes to yellow, then discard it.

7. SAMPLE COLLECTION AND STORAGE

7.1 Collect blood samples according to the standard laboratory procedures using sterile serum preparation tubes or serum separator tubes (SST) for the preparation of serum.

7.2 Specimen storage: Serum samples can be stored temporarily at 2-8℃ before assay, or frozen at -20℃ or colder for longer term storage.

7.3 Use glucan-free EP tubes to store Serum at -20℃.

7.4 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.

-To avoid air bubbles, reverse pipetting is recommended.

-Preheat the microplate reader and make it stable at 37℃.

-Note: Settings may vary with different instruments and software. In general, the following will apply: Set the platereader software to collect data in the Vmean mode. Check the software manual for the proper settings to ensure that the value calculated is the mean rate of optical density change for all of the datapoints gathered. Set the interval between instrument 'reads' to

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the minimum allowed by the software and instrument over the 40 minute period of the test. The software wavelength settings should be 405 nm minus the background at 490 nm. If dual wavelength reading is not available, read at 405 nm. The incubation temperature is to be set at 37°C. The plate shaking should occur, for 5 – 10 seconds, prior to the commencement of reading. The curve fit setting should be “linear/linear” or equivalent. Reading should commence without any lag time.

8.1 Preparation of Standard Solution

8.1.1 One vial of Standard (R4) + 1.5mL of Diluent (R6). Vortex for at least 1 min and get Standard A.

8.1.2 Make serial dilution from Standard A to prepare standards B, C, D and E step bystep.

Standard ID	Concentration	Dilution	
Standard A	600 pg/mL	1.5 mL Diluent(R6) + Standard (R4)	Vortex: 1 min
Standard B	300 pg/mL	0.3 mL Diluent(R6) + 0.3 mL Standard A	Vortex: 30 sec
Standard C	150 pg/mL	0.3 mL Diluent(R6) + 0.3 mLStandard B	Vortex: 30 sec
Standard D	75 pg/mL	0.3 mL Diluent(R6) + 0.3 mLStandard C	Vortex: 30 sec
Standard E	37.5 pg/mL	0.3 mL Diluent(R6) + 0.3 mLStandard D	Vortex: 30 sec

Note: Vortex for at least 1 min to solve standard (R4). Do not touch inside of the caps of all vials and tubes to avoid contamination.

(1) All the above solutions are prepared for the standard curve. As all the control and samples are treated with treatment solution at 20uL + 40uL, this means the control and samples are diluted with 1:3. 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

- 1 vial of Control(R5) + 1.5mL of Diluent (R6). Vortex for at least 1 min.

Note: As in total 5 individual vials of control are provided for each kit, freezing used controls are not recommended. Fresh controls should be used for each run.

8.3 Pipetting of negative control

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

8.4 Pipetting of Standard Solutions

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

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8.5 Pipetting of Serum and addition of Pre-treatment Solutions

8.5.1 Pipette 20μL of serum/positive control into microplate wells.

8.5.2 Add 40μL of Treatment Solution R3 into the microplate wells that contain 20uL of serum sample /positive control in Step 8.5.1. Seal the unused treatment solution vial with parafilm and store at 2-8℃.

8.5.3 Shake for 5-10 seconds and incubate the microplate at 37℃ for 10 mins.

8.6 Addition of freshly prepared Main Reagent

8.6.1 It is recommended to resolve the Main Reagent during incubation of samples.

8.6.2 Main Reagent (R1) + 2.6mL of Reconstitution Solution (R7) , mix gently and thoroughly. Do not vortex.

8.6.3 One vial of Main Reagent (R1) are enough for 24 wells. If the tests need more than 24 wells in a run, then mix all vials of Main Reagent(R1) into one vial before addition.

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

8.6.5 Shake the plate for 5-10 seconds.

8.6.7 Read OD value at **37 ℃ kinetically** at 405nm and 490nm for 40 mins.

9. DATA ANALYSIS

Take "**Mean Slope OD/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.

A reader software with linear regression could be used for calculation.

10. QUALITY CONTROL

10.1 The Mean slope OD/min of negative control must be less than Standard E. 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 r^2 must be > 0.980.

10.4 The calculated concentration of Positive Control must fall within the range as shown on the Control Range Sheet which is put in the kit.

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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%

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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 disposable filter 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.

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15. REFERENCES

[1] Mori, T, Ikemoto, H, et al: Evaluation of Plasma (1-3)-β-D-Glucan Measurement by the kinetic Turbidimetric Limulus Test, for the Clinical Diagnosis of Mycotic Infection. Eur. J. Clin. Chem. and Clin. Biochem. 35, 553-560 (1997).

[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
	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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