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TECO®

Fungus (1-3)-β-D-Glucan Assay

Instructions for use
English

CE

Catalog Nr. TE1068
UDI-DI 7640146270085

TE1068_AA-E_11/2024

TECOmedical AG

www.tecomedical.com

Symbol Description

REF
TE1068

LOT
Lot number

UDI-DI
Unique Device Identifier

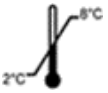
IVD
In Vitro Diagnostics


Manufacturer

Read electronic kit instructions


96
96 tests


Expiry date



Storage temperature

CE

Professional Use only

TECO*medical* AG
A Eurobio Scientific Company
Gewerbestrasse 10
4450 Sissach, Switzerland
Phone +41 61 985 81 00
info@tecomedical.com
www.tecomedical.com

EC REP &
Eurobio Scientific SA
7 avenue de Scandinavie
ZA de Courtaboeuf
91940 Les Ulis, France
Phone +33 1 69 79 64 80
reglementaire@eurobioscientific.com

Any serious incident that has occurred in relation to this product shall be reported to TECOmedical AG and the competent authority of the Member State in which the user and/or the patient is established

TECO® Fungus (1-3)-β-D-Glucan Assay

CONT **Reagents and Materials Supplied:**

SYMBOL	DESCRIPTION	FORMAT
R1	**Main Reagent** G Factor and Proclotting Enzyme Lyophilized	**4 x 2.6 ml**
R3	**Treatment Solution** Ready to use	**4 x 1.5 ml**
R4	**Standard** (1-3)- β-D-Glucan Lyophilized	**5 x 1.5 ml**
R5	**Control** (1-3)- β-D-Glucan Lyophilized	**5 x 1.5 ml**
R6	**Diluent** Deionized water Ready to use	**4 x 8 ml**
R7	**Reconstitution Solution** Tris-HCl Buffer Ready to use	**4 x 3 ml**
R8	**96-well plate** 12 break apart strips of 8 wells (12 x 8 in total), in a frame Ready to use.	**1 plate**
	**eIFU indicator** Login address for electronic kit instructions	

Storage

Store kit at 2–8°C until expiration date.

Intended Use

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.

Clinical Use

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 catheters, 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.
(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. For this reason, detection of (1-3)-β-D-Glucan in human serum offers a diagnostic reference for invasive fungal diseases.

Limitations

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.

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

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

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 (streptosepticaemia in particular).
f. Subjects who receive treatment with some antitumor drugs (lentinan and schizophyllan).
g. Subjects who receive treatment with sulfonamides.

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

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[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.

Assay Principle

The TECO Fungus (1-3)-β-D-Glucan Assay is based on pathways as shown below (Figure1). 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.

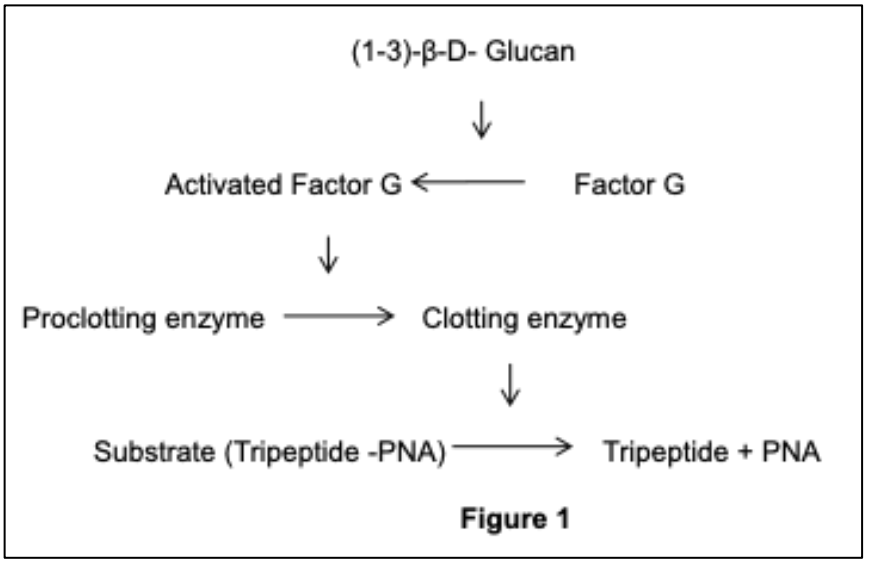


Figure 1

Materials Required and not Supplied

- Disposable gloves (powder free)
- Pipettes 10 – 1000 µl
- Multichannel pipettes 40 – 100 µl
- Pyrogen-free transfer tubes (for dilution of sample or standard)
- Centrifuge
- Vortex mixer
- Timer
- Spectrophotometer with kinetic reading (405nm and 490nm) and 37°C incubation function

IMPORTANT NOTES:
Transfer tubes and pipette tips must be pyrogen-free
Change pipette tips with every pipetting step, also between duplicates

The following materials are recommended for use with the TECO Fungus (1-3)-β-D-Glucan Assay

Safe Lock Tubes 1,5 ml	Art.No. DNK-1401-1-4
Eppendorf BIOPUR 2-200 ul	Art.No. 0030075030
Eppendorf BIOPUR 50-1000ul	Art.No. 0030075250

Warnings and precautions

This kit is intended for in vitro diagnostic use by professional persons only.

Follow the instructions carefully

Observe expiration dates stated on the labels. Allow the reagents to reach room temperature for 30 minutes and use reagents immediately after these 30 minutes.
Refer to "Materials Safety Data Sheet" for more detailed safety information.

TECOmedical AG is not liable for loss or harm caused by non-observance of the kit instructions.

1. For in vitro diagnostic use
2. **Working area must be cleaned before every assay to avoid contamination**
3. Prevent samples and reagents from contamination of fungi and bacteria
4. Treat all specimen samples and controls provided in the kit as potentially biohazardous material Follow General Precautions when handling contents of this kit and any patient samples
5. Main reagent **R1** and standard solutions should be prepared immediately before use
6. **Whilst preparing the standard curve do not touch the inside of the caps of all vials and tubes to avoid contamination**
7. Mix all reagents thoroughly before use
8. Disposal of containers and unused contents should be done in accordance with federal and local regulatory requirements
9. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label
10. Use of multichannel or repeat pipettors is recommended to ensure the timely delivery of liquids.
11. Store assay reagents as indicated. If the color of the frozen opened Main reagent changes to yellow, the reagent is invalid and should be discarded
12. Test each sample in duplicate

Preparation and stability of reagents

R1 Main Reagent

G Factor and Proclotting enzyme
4 vials of 2.6 ml, lyophilized
Reconstitute each vial with 2.6 ml of Reconstitution Solution **R7**
Mix carefully and thoroughly, do not Vortex
Store at 2–8 °C until expiration date or 5 days immediately after use at -20°C
Only one freeze – thaw cycle possible

R3 Treatment Solution

Alkaline solution
4 vials of 1.5 ml, ready to use, each vial is sufficient for treatment of 30 samples
Store at 2–8°C until expiration date

R4 Standard Solution

(1-3)- β-D-Glucan
5 vials of 1.5 ml, lyophilized
Reconstitute 1 vial of Standard **R4** with 1.5 ml of Diluent **R6**
Vortex for at least 1 minute to get Standard A.
Store at 2 – 8°C until expiration date, discard after use

Make serial dilutions from Standard A to prepare Standards B to E and vortex **30 seconds carefully between the steps** (See the Table below):

Standard ID	Concentration (pg/ml)	Dilution
A	600	1.5 ml Diluent **R6** +Standard **R4**
B	300	0.3 ml Diluent **R6** + 0.3 ml Std A
C	150	0.3 ml Diluent **R6** + 0.3 ml Std B
D	75	0.3 ml Diluent **R6** + 0.3 ml Std C
E	37.5	0.3 ml Diluent **R6** + 0.3 ml Std D

R5 Control

(1-3)- β-D-Glucan
5 vials of 1.5 ml, lyophilized
Reconstitute 1 vial of Control **R5** with 1.5 ml of Diluent **R6**.
Vortex for at least 1 minute.
Store at 2 – 8°C until expiration date, discard after use

R6 Diluent

Deionized Water
4 vials of 8 ml, ready to use.
Store at 2 – 8°C until expiration date

R7 Reconstitution Solution

Tris-HCl Buffer
4 vials of 3 ml, ready to use.
Store unopened at 2–8°C until expiration date, discard after use

Preparation and stability of samples

Collect blood samples according to the standard laboratory procedures using sterile preparation tubes or serum separator tubes (SST) for the preparation of serum. Avoid cross-contamination among samples. Sample labeling should be clear and correct without mistake.
Use glucan-free EP tubes for prolonged storage of serum at -20°C or less.

Sample Type

The assay is validated for serum.
Avoid Hemolysis, turbid sample with high lipid concentration, jaundice.

Sample transportation

Sample transportation should comply with national biosafety requirements. Transport and store samples in sealed tubes, unexposed to air.

Stability

72 hours at 2-8°C
12 months at -20°C
At least 5 freeze – thaw cycles

Preparation of spectrophotometer

Preheat the spectrophotometer and make it stable at 37°C. **Always check the programming of the spectrophotometer before use.**
Note: settings may vary with different instruments and software. In general, the following will apply:

Set the spectrophotometer software to collect data in the V_{mean} 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 the datapoints gathered. Set the interval between instrument 'reads' to the minimum allowed by the software and instrument over the 40 minutes 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**.**

Assay procedure

All determinations (controls and samples) should be assayed preferably in duplicate. When performing the assay, the standards, controls, and samples should be pipetted as fast as possible.

Allow all reagents to reach room temperature (20-25°C) for at least 30 minutes.

1. Allocate the wells of the Microtiter plate **R8** for standards, controls and samples
2. Pipette 60 µl of Diluent **R6** into the corresponding wells as negative control
3. Pipette 60 µl of Standard solutions (A, B, C, D and E) into the corresponding wells
4. Pipette 20 µl of serum samples or positive control into the corresponding wells
5. Add 40 µl of Treatment Solution **R3** into the microplate wells that contain 20 µl of serum samples or positive control
6. Shake for 5 -10 seconds and incubate the Microtiter plate at 37°C for 10 minutes
7. Add 100 µl of freshly reconstituted Main Reagent **R1** into each well
8. Shake the plate for 5-10 seconds
9. Read OD value at **37°C kinetically** at 405 nm and 490 nm for 40 minutes

Calculation of results

Establishing the Standard Curve
A calibration curve can be established by plotting the standard concentration on the x-axis against the Mean Slope OD/min on the y-axis. A linear regression curve fit should be used for data reduction.
The 1-3 β-D-Glucan concentration of the samples and control can be read off from this standard curve.

Typical results
The Mean slope OD/min of negative control must be less than Standard E. That indicates the test operation is free of contamination.

If there is a large deviation in the standard curve, it is recommended to repeat the test.
The square of correlation coefficient r^2 must be > 0.980.

For each assay the calculated concentration of positive control must be within the target range indicated on the label of Control **R5** bottle for each specific lot. If the value of the positive control is not within the limits of the target range, the assay results should be considered questionable and the samples should be tested again.

Interpretation of Results

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

Result < 70 pg/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.

Reference values

302 serum samples are measured with the TECO Fungus (1-3)-β-D-Glucan kit.

Results	Coincidence rate (CI)	95% confidence interval	Number of samples
Positive coincidence rate (Diagnostic sensitivity)	86.55%	79.27% - 91.55%	119
Negative coincidence rate (Diagnostic specificity)	89.07%	83.72% - 92.81%	183

Test performance

Precision
(Intra assay, N=80)

Sample	Mean (pg/ml)	SD	CV %
Reference sample	304.23	14.07	4.62

(Inter assay, N=20 in two different kit batches)

Sample	Mean (pg/ml)	SD	CV %
Reference sample	301.47	12.97	4.30

Accuracy

The accuracy of the TECOmedical Fungus (1-3)-ß-D Glucan Assay is evaluated by detecting the relative deviation at 200 pg/ml measured in triplicate in three different kit lots.

Kit	Mean value	Relative Deviation %
Lot 1	202.43	6.24
Lot 2	199.87	5.96
Lot 3	203.15	6.12

Interference

Controlled test of potential interfering substances in serum showed that here was no interference in the under-mentioned concentrations.

Substance	Concentration
Hemoglobin in hemolyzed samples	≤7.0 mg/ml
Triglycerides in high-fat turbid samples	≤5.0 mmol/l
Bilirubin in jaundice samples	≤170 µmol/l

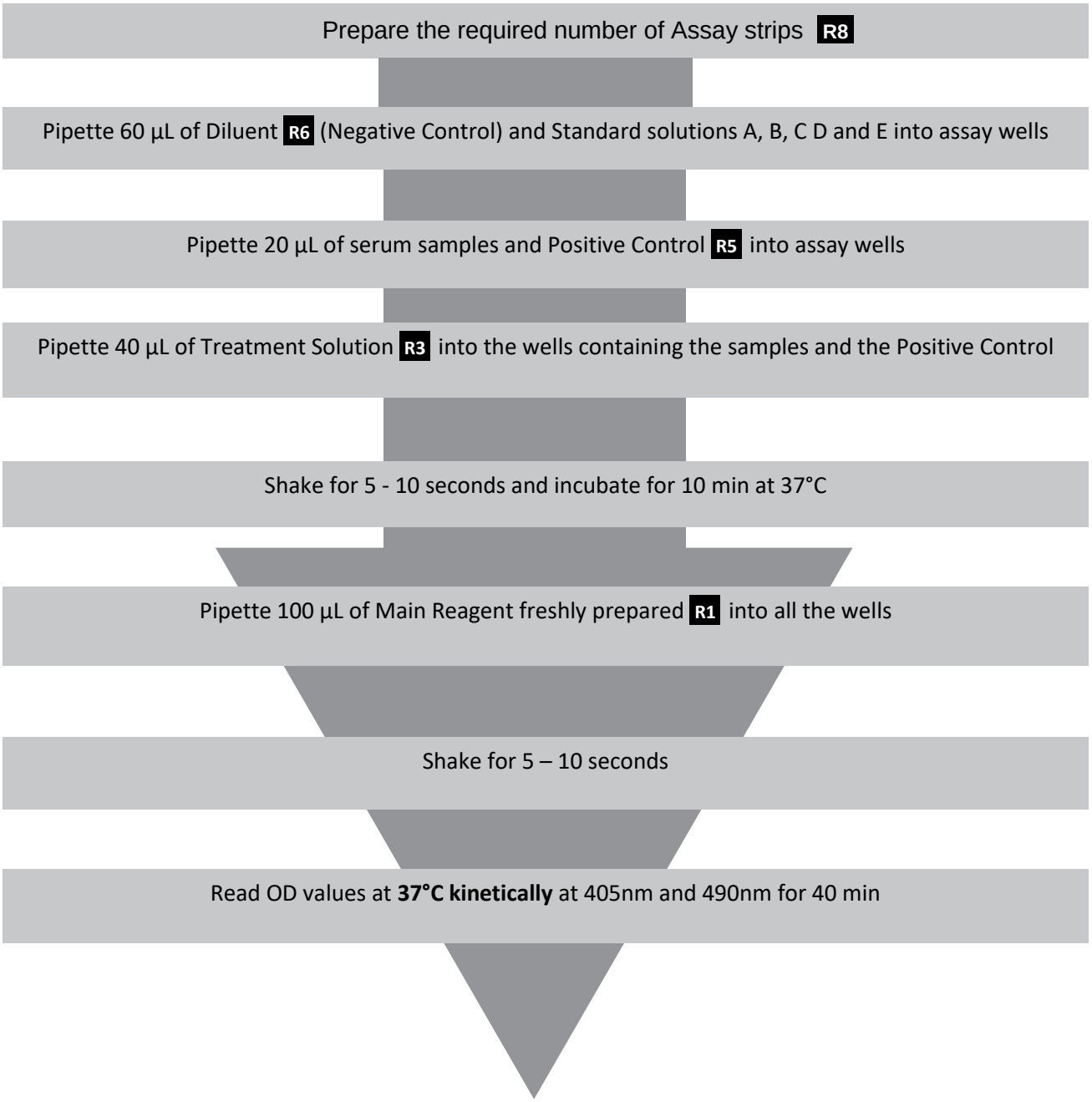
Cross reaction

Cross reactant	Concentration
Endotoxin	≤1.0 EU/ml

TECO® Fungus (1-3) -β-D-Glucan Assay

Assay Procedure – Quick Guide

- Bring samples and reagents to room temperature (20-25°C) for at least 30 min. Mix the samples well.
- Reconstitute the Control and prepare the Standard solutions.
- Preheat the spectrophotometer.
- Main reagent **R1** and standard solutions should be prepared immediately before use



Please read Kit instruction before using the Quick Guide