

#### Intended use

The β-Glucan Test is an *in vitro* assay for the quantitative determination of β-glucan in serum or plasma.

#### Summary and explanation of the test

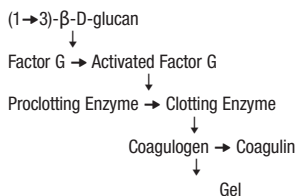
(1→3)-β-D-glucan is a fungal cell wall component. The β-Glucan Test is a helpful diagnostic marker for many invasive fungal infections.

#### Principle of the method

In order to suppress endotoxin activity, the sample is added to the pretreatment solution and heated.

When a pretreated sample containing (1→3)-β-D-glucan is mixed with the LAL Reagent, the cascade reaction below is triggered by Factor G. This reaction induces turbidity along with gelation.

The amount of (1→3)-β-D-glucan in the sample can be calculated based on a proportional relationship between the amount of (1→3)-β-D-glucan and the gelation time which is needed to reach a defined turbidity with a known amount of (1→3)-β-D-glucan.



#### Reagents and contents

R1: Pretreatment Solution  
R2: LAL Reagent (for 0.2 mL)  
(Limulus amoebocyte lysate, Albumin (human))

Calibration card, cap opener.

#### Reagent preparation

- R1: Use Pretreatment Solution as supplied. After opening the reagent, it is recommended to use it immediately and do not store it.  
R2: Use LAL Reagent as supplied. After opening the reagent, it is recommended to use it immediately and do not store it.

#### Specimen collection and preparation

Use serum or plasma as a specimen.

Assay samples immediately after collection.

It is recommended that specimen collection is carried out in accordance with local and national regulations. Since all specimens are potentially infectious, they should be handled in accordance with local or national regulations relating to the safe handling of such materials.

The stability of (1→3)-β-D-glucan is summarized below (own data). The stability of (1→3)-β-D-glucan in the sample depends on the sample's characteristics.

Storage temperature	Stability of (1→3)-β-D-glucan in a sample
-80 °C	Stable up to 30 days
4 °C	About 6 % decrease after 3 days
25 °C	About 20 % decrease after 2 days

#### Test procedure

##### 1) Calibration

See calibration data sheet for gelation time and concentration as accessory in the β-Glucan Test R2: LAL Reagent.

**Traceability:** The β-glucan concentration was determined by measuring a calibrator with the β-Glucan Test reagent and the Wako 1st β-Glucan standard. Wako 1st β-Glucan standard was prepared using lentinan.

##### 2) Materials/equipment required, separately available

- Toxinometer MT-6500.
- Thermostation TS-70/16
- Aluminum Cap
- β-Glucan Sample Diluent (separately available)
- LAL Control (separately available)
- Tip: BC Tip EXT / BC Tip 1000-R
- Cooling Station

##### 3) Materials/equipment required, not supplied

- Vortex mixer
- Pipette
- Ice box

##### 4) Measurement procedure

###### <Input calibration data>

Enter calibration data by reading the QR code printed on the calibration card with the reader connected to the Toxinometer MT-6500.

In case of QR code failure the calibration data printed on the calibration card can be entered manually. Refer to the manual of the instrument.

###### <Pretreatment for specimen>

Add 0.1 mL of heparinized plasma or serum to 0.9 mL of R1: Pretreatment Solution and mix well. Heat at 70 °C for exactly 10 minutes, followed by cooling with ice immediately for at least 3 min.

###### <Standard operation procedure>

	Sample (serum or plasma)	Positive control (LAL control)	Negative control (dissolution buffer of LAL control)
R2: LAL Reagent (for 0.2 mL)	Pretreated sample (for 0.2 mL)	Pretreated positive control (for 0.2 mL)	* Negative control (0.2 mL)

Measure time for gelation (Tg) by Toxinometer MT-6500 (37 °C, medium wavelength 660 nm.)

\*Do not use R1: Pretreatment Solution for the negative control

Definition of time for gelation (Tg): required reaction time until 92 % or less transmittance ratio is reached.

###### <Using Toxinometer MT-6500>

- 1) Prepare Toxinometer MT-6500 according to operation procedure in instruction manual. Check temperature at 37 °C (±0.5).
- 2) Use cap opener to open R2: LAL Reagent by twisting it slowly to an angle of 30–40 degrees in order to remove Aluminum Cap and rubber cap. Cover LAL tube with Aluminum Cap.
- 3) Confirm lot identity between calibration data and LAL.
- 4) Add 0.2 mL of pretreated sample to R2: LAL Reagent (for 0.2 mL) and mix it by vortex mixer for a few seconds after confirming visually that LAL is completely dissolved. Insert the LAL tube into Toxinometer MT-6500 measuring wells. The LAL should be inserted at position where green LED light is flashing.
- 5) Measurement is started automatically after inserting LAL tube. The green LED stops flashing and shows a constant green light.
- 6) The green LED turns off after completion of the assay.
- 7) Assay result of (1→3)-β-D-glucan is obtained from time for gelation (Tg) of specimen and attached calibration data. In the case of a very high BDG value (>600 pg/mL), dilute the pretreated sample. (Please refer to the package insert "β-Glucan Sample Diluent"). (1→3)-β-D-glucan concentration of the pretreated and diluted sample should be multiplied with the dilution factor.
- 8) Negative control and positive control should confirm following conditions:  
**Negative control:** Tg is 90 minutes and more. The dissolution buffer for LAL control should be used as negative control without sample pretreatment.  
**Positive control:** calculated value from calibration data is within the range of ±20 % of the known concentration. Positive control should be pretreated like specimen. LAL control should be used as positive control.

Contamination on equipment or at operation can be assumed when gelation time of negative control is less than 90 minutes or positive control result is 20 % and higher than known concentration. In such cases, re-examination is needed.

#### Expected values

Cut-off value: 7 pg/mL (as (1→3)-β-D-glucan)<sup>(1)(1)(3)</sup>

#### Performance characteristics

##### Sensitivity

- a) When water (0 pg/mL of (1→3)-β-D-glucan) is assayed, the gelation time is 90 minutes and above.
- b) When standard solution ((1→3)-β-D-glucan 3.3 pg/mL) is assayed, the time for gelation ranges from 25 to 55 minutes.

##### Specificity

When a sample of known concentration is assayed, the assay value is within the range of ±20 % of the known concentration.

#### Precision

##### [Within-run precision]

Below are representative data of the within-run precision. The results of CV % for each plasma sample measured in 21 replicates ranged from 3.4 % to 4.7 %

Sample no.	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	11.8	78.4	373.8
SD (pg/mL)	0.40	2.83	17.55
CV (%)	3.4	3.6	4.7

##### [Total precision]

Below are representative data of total precision data. All data were collected in accordance with CLSI protocol EP5-A3.

Sample no.	Sample 1	Sample 2	Sample 3
Total mean (pg/mL)	10.6	74.1	392.2
Total precision SD (pg/mL)	0.7	4.9	25.6
Total precision CV (%)	6.4	6.6	6.5

#### Accuracy

The accuracy of this method was determined by a recovery study.

##### Plasma specimen 1

Added (pg/mL)	0.0	75.0	150.0	300.0
Measured	10.0	73.2	134.3	275.9
	10.4	73.2	134.3	250.5
	10.2	67.4	134.3	290.0
mean (pg/mL)	10.2	71.3	134.3	272.1
Obtained (pg/mL)		61.1	124.1	261.9
Recovery %	-----	81.5 %	82.7 %	87.3 %

##### Plasma specimen 2

Added (pg/mL)	0.0	75.0	150.0	300.0
Measured	73.2	144.6	239.0	378.2
	73.2	139.6	228.2	400.2
	71.2	139.3	228.2	378.2
mean (pg/mL)	72.5	141.1	231.8	385.5
Obtained (pg/mL)		68.6	159.3	313.0
Recovery %	-----	91.5 %	106.2 %	104.3 %

##### Plasma specimen 3

Added (pg/mL)	0.0	75.0	150.0	300.0
Measured	191.3	250.5	357.8	478.1
	191.3	250.5	338.9	478.1
	183.5	250.5	338.9	478.1
mean (pg/mL)	188.7	250.5	345.2	478.1
Obtained (pg/mL)		61.8	156.5	289.4
Recovery %	-----	82.4 %	104.3 %	96.5 %

The recovery rate of (1→3)-β-D-glucan is 81.5 %–106.2 % in the concentration ranges shown in the above table.

#### Linearity

(1→3)-β-D-glucan concentrations in specimens up to 600 pg/mL were linear in the evaluation. In the case of a very high BDG value (>600 pg/mL), dilute the pretreated sample. Please refer to the package insert "β-Glucan Sample Diluent".

#### Correlation<sup>(8)(13)</sup>

	Fungitell		Total	
	Positive	Negative		
WAKO Serum	Positive	127	3	130
	Negative	10	182	192
Total	137	185	322	

Percent positive agreement = 92.7 %  
Percent negative agreement = 98.4 %  
Overall agreement = 96.0 %

	Fungitell		Total	
	Positive	Negative		
WAKO Plasma	Positive	90	4	94
	Negative	1	64	65
Total	91	68	159	

Percent positive agreement = 98.9 %  
Percent negative agreement = 94.1 %  
Overall agreement = 96.9 %

#### Interfering substances

Bilirubin and hemolysis do not have significant influences on the assay.

#### Warnings and precautions

- For *in vitro* diagnostic use.
- The usage and application of this test is reserved for professional use only. Please refer to respective national and local regulations and legislation.
- Not to be used internally in humans or animals.
- If the reagents come in contact with the mouth, eyes, or skin, wash off immediately with a large amount of water. Consult a physician if necessary.
- In the case of using a glass pipette, do not pipette by mouth, but use a safety pipette.
- Be careful not to cut yourself with the Aluminum Cap when removing it from the vial.
- The R2: LAL Reagent contains materials of human origin. The materials have been tested and found negative for HBsAg, anti-HIV-1/HIV-2 antibodies, and anti-HCV antibody. Since the risk of infection cannot be excluded with certainty, the products must be handled as potentially biohazardous like patients' sera.
- Dispose of reagents according to your local or national regulations.

**β-Glucan Test R1 contains components classified as follows according to Regulation (EC) No. 1272/2008. Please note the safety data sheet!**

**Signal word:** Warning

#### Pictograms:



**Hazard statements:** H317..... May cause an allergic skin reaction.

#### Precautionary statements:

P280..... Wear protective gloves/protective clothing/eye protection/face protection.  
P303+P361+P353..... IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water (or shower).  
P308+P313..... IF exposed or concerned: Get medical advice/attention.

#### Hazardous ingredients

**for labelling:** 2-methylisothiazol-3(2H)-one

#### <Precautions for procedure>

- As an anticoagulant, heparin is recommended in the case of (1→3)-β-D-glucan assay in plasma. For plasma separation by centrifuging, it should be done between 2–10 °C, for 40 sec at 3,000 rpm with a rotor radius of 10 cm, or centrifuging for 10 minutes with 150 g\*. \*1,200 rpm with a rotor radius of 10 cm.
- Vacuumed blood collection tube must not be contaminated with (1→3)-β-D-glucan. Blood collection should be done carefully to avoid contamination with (1→3)-β-D-glucan.
- Pretreatment should be done immediately after collection. Keep the specimen in a container without contamination of (1→3)-β-D-glucan and freeze at -80 °C for storage if analysis is not immediately possible. The storage period shall be within one month
- Observe specified reaction temperature and reaction time.
- This assay may be influenced by contamination from equipment used or other contamination by handling errors. Proper equipment should be used and attention should be given to avoid contamination during the operation.
- Commercial vacuumed blood collection tubes and tips or glass equipment for dispensing should be used after exclusion of contamination with (1→3)-β-D-glucan.
- Be careful to avoid contamination of (1→3)-β-D-glucan during assay operation.
- The vial is sealed at reduced pressure. Slowly remove the stopper to avoid blowing the powder out of the vial.
- Do not touch or soil the bottom of LAL tube because this part is used for photometric measurement by Toxinometer.
- Before setting the LAL tube into Toxinometer, check the absence of bubbles in the mixture. In the case of bubbles, remove the bubbles by flicking at bottom of tube.
- If the measured value exceeds the measurable range, dilute pretreated mixture with β-Glucan Sample Diluent, repeat assay and multiply the obtained result by dilution factor.
- If the mixture shows significant turbidity, centrifuge pretreated mixture at 3,000 rpm for 20 minutes and use the supernatant as pretreated sample.

#### <Precautions for assays>

- Store the reagents under the specified conditions. Do not use reagents after the expiration date stated on each reagent container label.
- Do not use reagents which were frozen in error. Such reagents may give false results.
- After opening the reagents, it is recommended to use them immediately and do not store them.
- Do not use the containers and other materials in the package for any other purposes.
- Do not use the reagents described above for any purpose other than described herein.
- Do not use the reagents described above in any procedures other than those described herein. Performance cannot be guaranteed if the reagents are used in other procedures.
- Operate the instruments according to operation manuals under appropriate conditions. Consult the instrument manual for details.

#### <Precaution for determination/results and diagnosis>

- Some specimens show non-specific turbidity during the assay, which may give wrong results. Confirm presence or absence of non-specific turbidity by reaction time course or dilution test if a result gives rise to suspicion.
- Specimens from dialysis patients who have been treated with cellulose-based dialysis membranes or patients who have been treated with (1→3)-β-D-glucan-based drugs from mushrooms, such as lentinan or similar substances, may give false positive results.
- (1→3)-β-D-glucan concentration may rise transiently after surgery.
- High levels of endotoxin may give positive errors.
- The results should be used in connection with medical assessment and patients' symptoms.

#### <Precautions for disposal>

- Disposal of reagents must be done according to local or national regulations.
- All the devices including reagents and reagent bottles that come in contact with specimens should be considered potentially infectious.

#### Quality control

A quality control program is recommended for clinical laboratories. The analysis using FUJIFILM Wako's LAL control set with each assay is recommended for monitoring the performance of the procedure. The values obtained for the control should / must be within ±20 % of the assigned values.

#### Storage condition

Product	Storage condition
β-Glucan Test R2: LAL Reagent	Store between 2–10 °C
β-Glucan Test R1: Pretreatment Solution	Store between 2–10 °C

#### References

- 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 Infections, Eur. J. Clin. Chem. Biochem., 35, 553-560 (1997).
- Kakinuma, A., Asano, T., et al.: Biochem. Biophys. Res. Commun., 101, 434-439 (1981).
- Morita, T., Tanaka, S., Nakamura, T. and Iwanaga, S.: A New (1→3)-β-D-glucan Coagulation Pathway Found in Limulus Amebocytes. FEBS Lett., 129, 318-321 (1981).
- Nakamura, T., Morita, T., et al.: Japanese Society for Bacteriologies, 38, 781-803, (1983) (in Japanese).
- Stone, B. A. and Clarke, A. E.: Chemistry and Biology of (1→3)-β-D-glucans, 11-12, La Trobe University Press, Victoria, Australia (1992).
- Harada, K., Tsuchiya, M., et al.: 6th Endotoxin Symposium Proceedings, 7-12 (1993) (in Japanese).
- Harada, K., Tsuchiya, M., et al.: 40th Japanese Society for Symposium of Toxins Proceedings, 155-158 (1993) (in Japanese).
- Internal Data
- Lamoth, F., Cruciani, M., et al.: Clin. Infect. Dis., 54, 633-643 (2012).
- Dichtl, K., Seybold, U., et al.: J. Clin. Microbiol., 56, e00286-18 (2018).
- Dichtl, K., Seybold, U., et al.: Infection, 47, 217-224 (2019).
- Mercier, T., Guldentops, E., et al.: J. Clin. Microbiol., 57, e00322-19 (2019).
- De Carolis, E., Sanguinetti, M., et al.: PLoS One., 15, e0236095 (2020).
- Mercier, T., Guldentops, E., et al.: Clin. Infect. Dis., ciaa295 (2020).

#### Ordering information

Code	Product	Package
993-04201	β-Glucan Test R1: Pretreatment Solution	50 x 0.9 mL
997-04101	β-Glucan Test R2: LAL Reagent	50 x for 0.2 mL
995-04401	LAL Control R1: LAL Control (lyophilized) R2: Control dissolution buffer	10 x for 0.5 mL 10 x 2 mL
999-04301	β-Glucan Sample Diluent	10 x 0.9 mL
995-04901	Aluminum Cap	10 x 10 unit
995-05001	BC Tip EXT	100 tips
991-05101	BC Tip 1000-R	100 tips
993-04701	Toxinometer MT-6500	1 unit
999-04801	MT-6500 Extension Module	1 unit
993-03601	Thermostation TS 70/16	1 unit
998-22211	Cooling Station	1 unit