(1,3)-Beta-D-Glucan Detection Reagent Kit

GLUCATELL®

Manufactured by:
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Catalog#
GT002 - Kinetic assays; 110 tests
GT003 - With diazo-reagents for end-point assays; 55 tests
GT004 - Kinetic assays; 55 tests

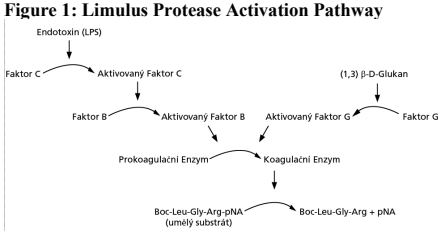
GLUCATELL® KITS ARE FOR RESEARCH USE ONLY AND ARE NOT TO BE USED FOR DIAGNOSTIC TESTING PURPOSES.

SUMMARY AND EXPLANATION OF GLUCATELL

Background

Horseshoe crabs form a blood clot to protect themselves against loss of blood and from invading microbes. Their blood contains a single cell type known as an amebocyte. Upon contact with bacterial endotoxins, the amebocytes degranulate and release zymogens involved in the clot forming pathway. These zymogens become active serine proteases in two ways: (1) endotoxins (lipopolysaccharides) of gram-negative bacteria activate Factor C, or (2) (1,3)-β-D-glucan activates Factor G (1,2). Both activation events result in clot formation when coagulogen is cleaved to coagulin by the clotting enzyme (Figure 1). The inclusion of a chromogenic peptide substrate in the reagent, permits spectrophotometric quantitation of the activated proclotting enzyme.

Figure 1: Limulus Protease Activation Pathway



Principles of the Glucatell Reagent

Amebocytes collected from the hemolymph of *Limulus* polyphemus, are washed and then lysed. This lysate is processed to remove Factor C making the lysate specific for (1,3)-β-D-glucan. Glucatell is then formulated as a chromogenic lysate by adding Boc-Leu-Gly-Arg-pNA. (1,3)-β-D-glucan in the test sample (or standard) activates Factor G, which then activates the proclotting enzyme. The clotting enzyme cleaves p-nitroaniline (pNA) from the chromogenic peptide substrate. The free pNA is measured at 405 nm (kinetic assay) or alternatively, the pNA is diazotized to form a compound that absorbs at 540-550 nm (endpoint assay).

Attention: Glucatell reagent includes processed Limulus amebocyte lysate from horseshoe crabs. Because its toxicity is unknown, exercise caution when handling this reagent.

The Glucatell kit is available for either an end-point or kinetic chromogenic assay, in a convenient microplate format. A microplate reader is required to perform the assay (incubating or non-incubating depending on the kit chosen).

MATERIALS SUPPLIED WITH THE 55 TEST KIT

1. 1 vial Glucatell Reagent
2. 1 vial Pyrosol Reconstitution Buffer*
3. 1 vial (1,3)-β-D-Glucan Standard
4. 1 bottle LAL Reagent Water*, 20 mL
5. Diazo Reagents (included with endpoint assay kits only)
   1 vial HCl
   1 vial Sodium Nitrite
   1 vial Ammonium Sulfamate
   1 vial NEDA (N-(1-Napthyl)ethylenediamine HCl)
   1 vial N-methyl-Pyrrolidinone
6. 1 microplate, 96-well*

Note: 20 mL Reagent Grade Water and LAL Reagent Water glass vials are equivalent.

MATERIALS SUPPLIED WITH THE 110 TEST KIT

Materials supplied with the 110 test kit are the same as those supplied with the 55 test kit. Two of each item are supplied in the 110 test kit. Kinetic method kits do not contain diazotization reagents.

STORAGE CONDITIONS

Store all reagents at 2-8°C in the dark. Reconstituted Glucatell reagent should be stored at 2-8°C and used within 2 hours. Alternatively, reconstituted Glucatell reagent can be frozen at -20°C for up to 20 days, thawed once and used. The diazo-reagents should be used the day they are prepared.

MATERIALS REQUIRED BUT NOT SUPPLIED

All materials and glassware must be free of interfering glucan. Glassware must be baked out at 235°C for 7 hours to be considered suitable for use. Purchase plastic supplies from a supplier that will certify that the material is free of interfering substances.

1. Pipette tips * (250 µL - Cat# PPT25; 1000 µL - Cat# PPT10)
2. Test tubes for sample dilutions (13 x 100 mm - Cat# TB013)
3. Glass pipettes
4. a. Kinetic assay - Incubating plate reader capable of reading at OD 405 nm with appropriate kinetic assay software or
   b. End-point assay - Plate reader capable of reading at 540-550 nm and a heating block.

** these products are certified free of interfering glucans*

PROCEDURE - END-POINT ASSAY (5 - 40 pg/mL RANGE)

Preparation of reagents

1. Preparation of standard solution: The β-glucan content is stated on the vial label. Add appropriate volume of LAL Reagent Water to the vial of glucan standard (Pachyman) to make a 100 pg/mL solution (see vial label for volume to add). Vortex for at least one minute to resuspend homogeneously.
2. Prepare a 40 pg/mL solution by mixing 600 µL LAL Reagent Water and 400 µL of glucan solution (1) in a glucan free tube.
   NOTE: Expel pipette tip contents slowly to ensure complete transfer.
3. Prepare a 20 pg/mL solution by mixing 400 µL LAL Reagent Water and 400 µL of glucan solution (2) in a glucan free tube.
4. Prepare a 10 pg/mL solution by mixing 400 µL LAL Reagent Water and 400 µL of glucan solution (3) in a glucan free tube.
5. Prepare a 5 pg/mL solution by mixing 400 µL LAL Reagent Water and 400 µL of glucan solution (4) in a glucan free tube.
6. Reconstitute one vial of Glucatell reagent with 2.8 mL of Pyrosol. Swirl the vial gently to dissolve completely (DO NOT VORTEX.)
   Use the reconstituted Glucatell reagent within 10 minutes or place it at 2-8°C and use within 2 hours.
7. Preparation of the diazo coupling reagents:
   a. Add the 1N HCl solution (vial 1A) to the sodium nitrite (vial 1),
   b. Add 4.0 mL of LAL Reagent Water to the ammonium sulfamate (vial 2).
   c. Add the contents of the of N-methyl-Pyrrolidinone (vial 3A) to the N-(1-Napthyl) ethylenediamine dihydrochloride (NEDA) (vial 3). These solutions should be used the same day.

Blank and standards are assayed in duplicate (or triplicate) as follows.

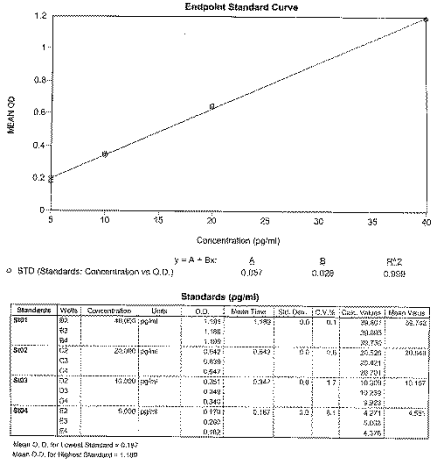
Duplicate scheme example:

1. Add 50 µL of the 40 pg/mL standard solution to wells B2, B3.
2. Add 50 µL of the 20 pg/mL standard solution to wells C2, C3.
3. Add 50 µL of the 10 pg/mL standard solution to wells D2, D3.
4. Add 50 µL of the 5 pg/mL standard solution to wells E2, E3.
5. Add 50 µL of LAL Reagent Water to wells F2, F3.

Assay procedure - Endpoint.

1. Add 50 µL of sample to the unknown wells.
2. Add 50 µL of Glucatell reagent to each well using a repeater pipette. Cover the plate with its lid and shake by tapping the edge. Place the plate in a heating block or a plate reader preheated to 37°C ± 1°C for the recommended incubation time (see Certificate of Analysis) for the curve chosen.
3. Stop the reaction by adding 50 µL of sodium nitrite (vial 1) with a repeater pipette. Then add in sequence 50 µL of ammonium sulfamate (vial 2), and then 50 µL of N-(1-Napthyl)ethylenediamine dihydrochloride (NEDA) (vial 3), using a new pipette tip each time. Color development is immediate. Place the uncovered plate in the microplate reader and read the optical density at 540-550 nm.
4. Use the plate reader software to plot a linear standard curve and to calculate the concentration of (1,3)-β-D-glucan in the unknowns.

Example:



PROCEDURE - KINETIC: RATE ASSAY (3.125 - 50 pg/mL RANGE)

Example of Preparation of Standard Curve

The glucan standard is to be reconstituted with Reagent Grade Water to provide a solution of 100 pg glucan/mL.

1. Dissolve the glucan standard with the appropriate volume of LAL Reagent Water (will vary for each lot, see vial label) to make a 100 pg/mL solution and vortex well.
2. Prepare a 50 pg/mL standard by mixing 400 µL LAL Reagent Water and 400 µL of solution (1) in a glucan free tube.
   NOTE: Expel pipette tip contents slowly to ensure complete transfer.
3. Prepare a 25 pg/mL standard by mixing 400 µL LAL Reagent Water and 400 µL of solution (2) in a glucan free tube.
4. Prepare a 12.5 pg/mL standard by mixing 400 µL LAL Reagent Water and 400 µL of solution (3) in a glucan free tube.
5. Prepare a 6.25 pg/mL standard by mixing 400 µL LAL Reagent Water and 400 µL of solution (4) in a glucan free tube.
6. Prepare a 3.125 pg/mL standard by mixing 400 µL LAL Reagent Water and 400 µL of solution (5) in a glucan free tube.
   Note: *The volume of standard in the kinetic assay (25 µL per well) is half the volume of the standard used in the endpoint assay.*

Negative controls and standards are assayed in duplicate (or triplicate) as follows.

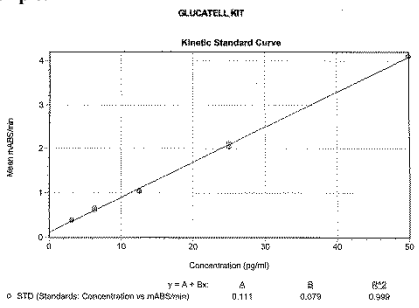
Triplicate scheme example:

1. Add 25 µL of the 50 pg/mL standard solution to wells B2, B3, B4.
2. Add 25 µL of the 25 pg/mL standard solution to wells C2, C3, C4.
3. Add 25 µL of the 12.5 pg/mL standard solution to wells D2, D3, D4.
4. Add 25 µL of the 6.25 pg/mL standard solution to wells E2, E3, E4.
5. Add 25 µL of the 3.125 pg/mL standard solution to wells F2, F3, F4.
6. Add 25 µL of LAL Reagent Water to wells G2, G3, G4.

Preparation/use of Glucatell lysate for kinetic assay procedure.

1. Set up the software to measure rate of change (mAbs/min).
2. Reconstitute one vial of Glucatell reagent with 2.8 mL of LAL Reagent Water and then add 2.8 mL of Pyrosol buffer. Swirl the vial gently to dissolve completely (DO NOT VORTEX.)
   Use the reconstituted Glucatell reagent within 10 minutes or place it at 2-8°C and use within 2 hours.
3. Add 25 µL of sample to the unknown wells.
4. Add 100 µL of Glucatell reagent to each well using a repeater pipette. Place the uncovered plate in the plate reader preheated to 37°C ± 1°C and shake for a minimum of 5 seconds before reading at 405 nm or 405 nm minus the 490 nm background (if available on the equipment and software being used). Set the software to take readings every 10 to 30 seconds for the recommended run time (see Certificate of Analysis) for the curve chosen.
5. Use the plate reader software to plot a linear standard curve and to calculate the concentration of (1,3)-β-D-glucan in the unknowns.

Example:



Standards (pg/ml)

Standards	Wells	Concentration	Units	mABS/min	Mean Time	Std. Dev.	C.V.%	Calc. Values	Mean Value
St01	B3	50.000	pg/ml	4.089	4.097	0.01	0.3	50.334	50.237
	B4			4.105				50.327	
St02	C3	25.000	pg/ml	2.025	2.078	0.05	2.5	24.247	24.786
	C4			2.130				25.313	
St03	D3	12.500	pg/ml	1.049	1.040	0.01	1.2	11.821	11.716
	D4			1.032				11.598	
St04	E3	6.250	pg/ml	0.658	0.642	0.03	4.3	6.390	6.605
	E4			0.627				6.809	
St05	F3	3.125	pg/ml	0.378	0.386	0.01	2.8	3.301	3.469
	F4			0.393				3.557	

Mean mABS/min for Lowest Standard = 0.505
Mean mABS/min for Highest Standard = 4.097

PROCEDURE - KINETIC: TIME OF ONSET ASSAY (3.125 - 100 pg/mL RANGE)

Glucatell may also be run in the kinetic mode using onset O.D., with a log - log plot of the onset times versus the standard concentrations. The incubation period using an onset O.D. of 0.03 is typically set for 60 minutes, for a curve consisting of at least three points within the limits of 500 and 3.125 pg/mL. Different values of onset O.D., incubation period, and standard curve range may be used, as long as criteria 1, 2, and 3 of the Test Performance Guidelines section are met.

Example of Preparation of Standard Curve

The glucan standard is to be reconstituted with LAL Reagent Water to provide a solution of 100 pg glucan/mL.

1. Dissolve the glucan standard with the appropriate volume (will vary for each lot, see vial label) of Reagent Water to make a 100 pg/mL solution and vortex well.
2. Prepare a 50 pg/mL standard by mixing 400 µL LAL Reagent Water and 400 µL of solution (1) in a glucan free tube.
   NOTE: Expel pipette tip contents slowly to ensure complete transfer.
3. Prepare a 25 pg/mL standard by mixing 400 µL LAL Reagent Water and 400 µL of solution (2) in a glucan free tube.
4. Prepare a 12.5 pg/mL standard by mixing 400 µL LAL Reagent Water and 400 µL of solution (3) in a glucan free tube.
5. Prepare a 6.25 pg/mL standard by mixing 400 µL LAL Reagent Water and 400 µL of solution (4) in a glucan free tube.
6. Prepare a 3.125 pg/mL standard by mixing 400 µL LAL Reagent Water and 400 µL of solution (5) in a glucan free tube.
   Note: *The volume of standard in the kinetic assay (25 µL per well) is half the volume of the standard used in the endpoint assay.*

Negative controls and standards are assayed in duplicate (or triplicate) as follows.

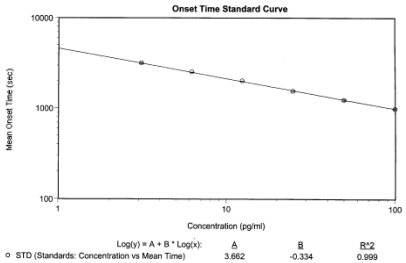
Triplicate scheme example:

1. Add 25 µL of the 100 pg/mL standard solution to wells B2, B3, B4.
2. Add 25 µL of the 50 pg/mL standard solution to wells C2, C3, C4.
3. Add 25 µL of the 25 pg/mL standard solution to wells D2, D3, D4.
4. Add 25 µL of the 12.5 pg/mL standard solution to wells E2, E3, E4.
5. Add 25 µL of the 6.25 pg/mL standard solution to wells F2, F3, F4.
6. Add 25 µL of the 3.125 pg/mL standard solution to wells G2, G3, G4.
7. Add 25 µL of LAL Reagent Water to wells B5, B6, B7.

Preparation/use of Glucatell lysate for kinetic assay procedure.

1. Set up the software to measure time of onset at 0.03 O.D. units. Onset time is defined as the interval (seconds) required for the reaction mixture to achieve a pre-set optical density.
2. Reconstitute one vial of Glucatell reagent with 2.8 mL of LAL Reagent Water and then add 2.8 mL of Pyrosol buffer. Swirl the vial gently to dissolve completely (DO NOT VORTEX.)
   Use the reconstituted Glucatell reagent within 10 minutes or place it at 2-8°C and use within 2 hours.
3. Add 25 µL of sample to the unknown wells.
4. Add 100 µL of Glucatell reagent to each well using a repeater pipette. Place the uncovered plate in the plate reader preheated to 37°C ± 1°C and shake for a minimum of 5 seconds before reading at 405 nm or 405 nm minus the 490 nm background (if available on the equipment and software being used). Set the software to take readings every 10 to 30 seconds for approximately 60 minutes at 37°C.
5. Use the plate reader software to plot a log-log standard curve and to calculate the concentration of (1,3)-β-D-glucan in the unknowns.

Example:



Standards (pg/ml)

Standards	Wells	Concentration	Units	Onset Time	Mean Time	Std. Dev.	C.V.%	Calc Value	Mean Value
St02	B3	100.0000	pg/ml	980	999	17.6	1.8	102.3680	96.7428
	C3			1015				92.1542	
	D3			1001				96.0689	
St03	B4	50.0000	pg/ml	1211	1234	20.2	1.6	54.3048	51.2875
	C4			1246				49.8625	
	D4			1246				49.8625	
St04	B5	25.0000	pg/ml	1554	1542	26.5	1.7	25.7289	26.3163
	C5			1512				27.9295	
	D5			1561				25.3849	
St05	B6	12.5000	pg/ml	1988	2002	24.2	1.2	12.3033	12.0474
	C6			1988				12.3033	
	D6			2030				11.5585	
St06	B7	6.2500	pg/ml	2520	2508	24.2	1.0	6.0471	6.1489
	C7			2520				6.0471	
	D7			2478				6.3594	
St07	B8	3.1250	pg/ml	3066	3131	70.5	2.3	3.3607	3.1550
	C8			3122				3.1834	
	D8			3206				2.9400	

Mean Onset Time for Lowest Standard = 3131
Mean Onset Time for Highest Standard = 999

iLUCATELL 1 HOUR ONSET TIME.pd1

TEST PERFORMANCE GUIDELINES

The following are guidelines for the evaluation of proper performance and results when using the Glucatell reagent. The performance criteria presented represent the minimum that users should accept for valid test results.

1. Correlation Coefficient

It is recommended that users obtain a correlation coefficient of 0.980, or higher, in the performance of either the endpoint assay or the kinetic assay. Failure to achieve an |r| value of 0.980 may be indicative of a problem of technique or materials contamination.

2. Standard Curve Glucan Concentration Recovery

Users should compare the calculated values of their standard curve glucan concentrations to the expected values. Average recovered values should be within the parameters established by the laboratory.

3. Spike Recovery

Users should expect to be able to recover product spikes within the range of 50% to 200% of the expected value. Failure to achieve spike recovery within these limits may indicate interference (enhancement or inhibition). If interference is observed, suitable measures may include sample dilution to overcome the interference.

4. pH Range for the Reaction

The buffer used to reconstitute the Glucatell reagent is 0.2*M* Tris, pH 7.4 at 37°C. If the sample to be analyzed will alter the pH significantly, it will be necessary to dilute or to adjust the pH close to a value that will allow product spike recovery to within the 50% to 200% range.

5. Biological Samples

Samples containing serine proteases must be inactivated to prevent them from interfering with the assay.

PRECAUTIONS

1. The Glucatell kit is intended for research purposes only. Not for in-vitro diagnosis of humans or animals.
2. The reconstituted Glucatell reagent should be used at one time or frozen at -20°C for up to 20 days. Once thawed, this reagent cannot be refrozen.
3. The reconstituted glucan standard should be stored at 2-8°C and used within 3 days. This standard can be stored at -20°C for up to 10 days in small quantities—once thawed it cannot be refrozen.

REFERENCES

1. Iwanaga, S., Morita, T., Nakamura, T., and Aketagawa, J. (1986) The hemolymph coagulation system in invertebrate animals. J. Protein Chem 5: 255-268.
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3. Saito, H., Yoshioka, Y., Uehara, N., Aketagawa, J., Tanaka, S., and Shibata, Y. (1991) Relationship between conformation and biological response for (1,3)-β-D-glucans in the activation of coagulation factor G from Limulus amebocyte lysate and host-mediated antitumor activity. Demonstration of single-helix conformation as a stimulant. Carbohydrate Res. 217:181-190.
4. Aketagawa, J., Tanaka, S., Tamura, H., Shibata, Y., and Saito, H. (1993) Activation of Limulus coagulation factor G by several (1,3)-β-D-glucans: Comparison of the potency of glucans with identical degree of polymerization but different conformations. J. Biochem 113:683-686.