β-GLUCAN
(Yeast and Mushroom)

VALIDATION REPORT

SKU: 700004358
K-YBGL

09/25

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INTRODUCTION:

The β-Glucan Assay Kit (Yeast and Mushroom) is designed for the indirect measurement of 1,3:1,6-β-glucan in a wide range of sample types, including mushroom, yeast, and algae preparations that may contain starch, maltodextrins, glycogen, sucrose, and trehalose. Data presented in this report validates that this method is fit for the purpose intended.

RECOMMENDATIONS FOR ANALYSIS:

Please reach out to your local sales representative or to the technical team should you require any assistance, particularly in relation to assay troubleshooting, data analysis and additional matrix testing.

- This test should only be carried out by trained laboratory employees. The product instructions must be followed to help ensure an accurate result.
- The time of incubation with GOPOD reagent is not critical but should be at least 20 minutes. The colour formed should be measured within 60 min. The absorbance of the GOPOD reagent solution should be less than 0.05 when read against distilled water.
- With each set of determinations, reagent blanks and glucose standard (100 µg in quadruplicate) should be included. An optional sample blank may be included for highly coloured samples.
- With each set of determinations, analysis of the Yeast Glucan Control (**Bottle 5**) is recommended.
- To help ensure an accurate result it is recommended that the user experimentally determines the Glucose Hydrolysis Correction factor (HCF).
- Use of a repetitive pipettor is recommended to reduce the risk of pipetting error.
- The reagent blank value must be determined once for each set of determinations and subtracted from each sample result.
- Users should perform matrix validation experiments prior to routine use. This process will highlight any problematic matrices encountered.
- Use separate pipette tips for each sample extract and control solutions to reduce the risk of cross-contamination. Additionally, pre-flush the tip before pipetting.
- When testing solid samples, ensure a representative portion is homogenized before weighing.

EQUIPMENT (RECOMMENDED):

1. Glass test tubes (round bottomed, 16 x 100 mm, 14 mL capacity).
2. Glass Culture Tubes. - Screw cap tubes, (20 x 125 mm, e.g. Fisher Scientific® Cat no. FB59563) plus caps (e.g. Fisher Scientific Cat no. FB51355). Screw cap tubes, (16 x 125 mm, e.g. Fisher Scientific Cat no. FB59559) plus caps (e.g. Fisher Scientific Cat no. FB51354).
3. Boiling water bath.
4. Micro-pipettors (e.g. Gilson® Pipetman®).
5. Positive displacement pipettor (e.g. Eppendorf® Multipette® with Combitip®).
6. Magnetic stir plate plus stirrer bars (5 x 15 mm).
7. Analytical balance.
8. Microfuge centrifuge (capable of running at 15,000 rcf).
9. Disposable polypropylene microfuge tubes (2.0 mL capacity, e.g. BRAND™).
10. Spectrophotometer set at 510 nm.
11. Thermostated water bath (capable of maintaining temperatures between 30°C and 50°C).
12. Vortex mixer.
13. Centrifugal mill (capable of milling material to a 1.0 mm screen size).
14. Syringe filters (0.2 micron, e.g. AGILENT™ Nylon or equivalent).
15. Filter paper (e.g. Sartorius™ grade 292 or equivalent).
16. Volumetric flasks (100 mL capacity).

17. pH-meter.

SUMMARY OF PERFORMANCE DATA:

All testing was performed using the standard assay described in the product instruction document. Results are summarised in the table below:

β-GLUCAN	
Limit of Detection (LOD)	0.5 % (*w/w*) for solid samples 0.1 % (*w/v*) for liquid samples
Limit of Quantification (LOQ)	1.5 % (*w/w*) for solid samples 0.3 % (*w/v*) for liquid samples
Limit of Precision	2.13 % (*w/w*) for solid samples
Repeatability (%CV)	< 5 %
Matrix Interference	Over-recovery of β-glucan if sample contains cellulose, maltitol or any other interfering glucan that is not measured in the α-glucan detection method.

TOTAL GLUCAN	
GOPOD Working Range	0.04 - 1.0 g/L of D-glucose
F Factor	Experimentally determined by the user A value of 90 ± 5 was determined as part of this validation
Limit of Detection (LOD)	0.5 % (*w/w*) for solid samples 0.1 % (*w/v*) for liquid samples
Limit of Quantification (LOQ)	1.5 % (*w/w*) for solid samples 0.3 % (*w/v*) for liquid samples
Limit of Precision	2.10 % (*w/w*) for solid samples
Glucose Hydrolysis Correction Factor	Experimentally determined by the user A value of 1.05 ± 0.02 was determined as part of this validation
Recovery of Kit Control	95 % - 105 %
Repeatability (%CV)	< 5 %
Matrix Interference	No interference observed in any of the tested matrices.
Robustness – Hydrolysate Clarification	No statistically relevant difference observed between methods tested for clarification of hydrolysate (filter paper, syringe filter and centrifugation)
Robustness – Impact of sample colour	Including sample blanks and accounting for sample coloration had no significant impact on results, suggesting sample specific blanks are unnecessary under the recommended assay conditions
Robustness – Overnight Storage of reaction hydrolysates	Storing hydrolysates overnight at 4°C is not recommended without further validation

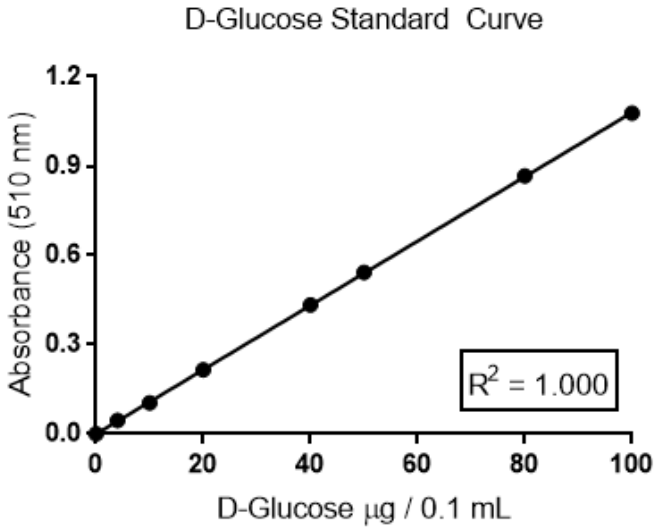
α-GLUCAN	
GOPOD Working Range	0.04 - 1.0 g/L D-glucose
F Factor	Experimentally determined by the user A value of 90 ± 5 was determined as part of this validation
Limit of Detection (LOD)	0.04 % (*w/w*) for solid samples 0.04 % (*w/v*) for liquid samples
Limit of Quantification (LOQ)	0.11 % (*w/w*) for solid samples 0.12 % (*w/v*) for liquid samples
Limit of Precision	0.60 % (*w/w*) for solid samples
Recovery of Kit Control	95 % - 105 %
Repeatability (%CV)	< 5 %
Target Efficiency of EnzyAlpha™	Starch, Glycogen, Maltodextrin, Sucrose and Trehalose are fully hydrolysed Maltitol, Microcrystalline Cellulose and Hydroxyethyl-cellulose are NOT hydrolysed by the enzyme mix.
Repeatability (%CV)	< 5%
Matrix Interference	No interference observed in any of the tested matrices
Robustness – Hydrolysate Clarification	No statistically relevant difference observed between methods tested for clarification of hydrolysate (filter paper, syringe filter and centrifugation)
Robustness – Impact of sample colour	Sample blanks are recommended for strongly colored samples
Robustness – Overnight Storage of reaction hydrolysates	Hydrolysates can be stored overnight at 4°C without affecting results for α-Glucan analysis, allowing the potential inclusion of a pause point in the α-Glucan workflow

GOPOD LINEARITY AND WORKING RANGE:

The working range for this kit was determined using the D-glucose standard provided in the kit. The D-glucose measurement (incubation with GOPOD Reagent) is linear between 4 and 100 µg of D-glucose per assay (0.04 - 1.0 g/L of D-glucose). Hydrolysate containing less than 0.04 g/L or more than 1.0 g/L will not fall within the acceptance criteria.

- It is recommended that the glucose standard is analyzed in quadruplicate.
- The F factor is calculated by dividing the ΔA obtained for the D-Glucose Standard (1.0 g/L – Bottle 4) by the expected concentration of D-glucose (100 µg). The F Factor should fall within the range of 90 ± 5.
- The absorbances of the samples assayed should not exceed that obtained for the D-glucose standard tested. If the sample absorbance exceeds the value obtained for the standard, dilute the sample further to achieve a suitable absorbance.
- The absorbance of the GOPOD blank solution should be less than 0.05 when read against distilled water.

Glucose Conc. µg/0.1 mL	ΔA
0	0
4	0.045
10	0.104
20	0.215
40	0.433
50	0.542
80	0.866
100	1.077



LIMIT OF DETECTION AND QUANTIFICATION:

The LOD is the lowest concentration of the analyte that can be detected by the method. This was determined by testing 20 replicates of the blank (i.e. without sample). The $\Delta A_{\text{Limit of Detection}}$ is calculated as 3.3 x s'0; where s'0 is the standard deviation of a number of samples $\Delta_{\text{Absorbance}}$ reading.

The LOQ is the lowest level at which the kit's performance is acceptably repeatable. This was determined by testing 20 replicates of the blank (i.e. without sample). The $\Delta A_{\text{Limit of Quantification}}$ is calculated as kQ x s'0; where s'0 is the standard deviation of a number of samples $\Delta_{\text{Absorbance}}$ reading. The IUPAC default value for kQ is 10.

The limit of detection and quantification for β-glucan were determined using the root sum of squares (RSS) method:

$$\text{LOD}_{\beta\text{-glucan}} = \sqrt{(\text{LOD}_{\text{Total}})^2 + (\text{LOD}_{\alpha})^2}$$

$$\text{LOQ}_{\beta\text{-glucan}} = \sqrt{(\text{LOQ}_{\text{Total}})^2 + (\text{LOQ}_{\alpha})^2}$$

	Limit of Detection			Limit of Quantification		
	ΔA	LOD % (*w/w*) Solid samples	LOD % (*w/v*) Liquid samples	ΔA	LOQ % (*w/w*) Solid samples	LOQ % (*w/v*) Liquid samples
Total Glucan	0.005	0.501	0.090	0.014	1.519	0.273
α-Glucan	0.004	0.037	0.008	0.012	0.112	0.024
β-Glucan	-	0.502	0.098	-	1.523	0.274

LIMITS OF PRECISION:

The Limits of Precision are the analyte concentrations at which it was experimentally determined that acceptable repeatability (%CV < 5 %) is routinely achieved. Limits of precisions were determined by measuring the lowest analyte concentrations, starting at LOQ level, for Total and α-Glucan methods. The limit of precision for β-glucan was determined from this data using the root sum of squares (RSS) method as described above.

Precision Limit Evaluation			
Total Glucan, % (*w/w*)	Total Glucan, %CV	α-Glucan, % *(w/w)*	α-Glucan, %CV
17.55	2.3	1.057	2.9
3.91	2.2	0.732	1.9
2.05	4.9	0.579	4.5
1.06	19.5	0.446	8.4

The lowest levels of Total and α-Glucan tested in the table above (values highlighted in red) did not fall within the repeatability acceptance criteria of < 5% CV and therefore the Limits of Precision were set as follows:

Analyte	Precision Limit
Total Glucan	2.10 % (*w/w*)
α-Glucan	0.60 % *(w/w)*
β-Glucan	2.13 % *(w/w)*

GLUCOSE HYDROLYSIS CORRECTION FACTOR (HCF):

An Hydrolysis Correction Factor (HCF) is required in Method A (Measurement of Total Glucan) to account for glucose loss resulting from the strong acid treatment used in the procedure. The HCF was experimentally determined by comparing the recovery of pure D-glucose (CAS: 50-99-7; >99% purity, not supplied) before and after acid hydrolysis. This correction factor is specific to the Total Glucan method and is not applied to the α-glucan method.

Sample	Analyst	Day	n, replicates	Hydrolysis Correction Factor (HCF)	Average HCF	%CV
Glucose	1	1	10	1.047	1.050	0.456
		2	10	1.054		
	2	1	10	1.055		
		2	10	1.043		

ENZYALPHA™ TARGET EFFICIENCY:

The α-glucan assay utilizes EnzyAlpha™, a proprietary enzyme mixture designed to hydrolyze specific α-glucan polysaccharides, including starch, maltodextrins, glycogen, sucrose, and trehalose. Additional potential interfering substances that contain glucan (Avicel, microcrystalline cellulose, hydroxyethyl cellulose, and maltitol) were evaluated due to their possible presence in yeast and mushroom-based products. Real samples were spiked with the potential interferents listed in the table below and degree of hydrolysis and sample recovery was assessed.

Sample	Total Glucan % *(w/w)*	α-Glucan % *(w/w)*	EnzyAlpha™ % Hydrolysis	Spiked Sample Recovery, %
Starch	87.08	86.52	99.3	100.28
Glycogen	79.20	80.25	101.3	100.25
Maltodextrin	85.12	81.57	95.8	101.14
Sucrose	43.69	46.96	107.5	98.42
Trehalose dihydrate	80.36	82.78	103.0	100.60
Maltitol*	44.77	0.53	1.2	98.73
Avicel PH-101 Cellulose	87.90	0.10	0.1	106.62
Microcrystalline Cellulose	78.37	0.11	0.1	105.12
Hydroxyethyl-cellulose	9.69	0.10	1.1	91.12

** Maltitol interference can be removed by using the optional application method outlined in the product instructions in which an additional enzymatic treatment is employed and all glucan arising from maltitol is captured in the a-glucan result.*

The results confirmed complete hydrolysis of starch, maltodextrins, glycogen, sucrose, and trehalose by EnzyAlpha™ in the α-glucan method, while Avicel, microcrystalline cellulose, hydroxyethyl cellulose, and maltitol remained unhydrolyzed under standard conditions. No interference was observed in the spiked samples, as glucose recovery remained within the defined acceptance criteria.

PRECISION AND REPEATABILITY

Precision is a measure of the variability in results obtained under different conditions, such as on different days and by different analysts. The acceptance criteria that was set as part of this validation was a coefficient of variation (%CV) of less than 5 %. The repeatability of the method was assessed using a variety of sample types, tested by two analysts over two days.

Sample Type	n, replicates	Total Glucan, % (*w/w* or *w/v*)	%CV	α-Glucan, % (*w/w* or *w/v*)	%CV	β-Glucan, % (*w/w* or *w/v*)	%CV
Algae product	12	89.54	0.75	0.06	71.8	89.48	0.7
Cordyceps Militaris	12	35.36	0.89	23.87	1.0	11.49	4.0
Mushroom Capsule	12	47.75	1.15	6.01	1.3	41.74	1.1
Mushroom Tincture	12	72.73	1.19	72.37	1.5	0.36	52.1
Mushroom Gummy*	12	35.81	1.11	30.86	1.9	4.96	5.2
Yeast Product	12	25.10	1.13	16.12	0.9	8.98	2.9

** The mushroom gummy was analysed using the optional application method outlined in the product instructions.*

Two of the samples (marked in red) exhibited elevated %CV values, which is attributed to their analyte concentrations being below the method's quantifiable precision limits. All other samples met the acceptance criteria of %CV < 5% for total glucan, α-glucan and β-glucan.

ROBUSTNESS

The robustness of the method was evaluated by deliberately varying method parameters to assess the method's reliability under typical usage conditions. Parameters such as the method used for hydrolysate clarification, the impact of sample colour on the results and the storage of the hydrolysates overnight at 4^{o}C prior to GOPOD measurement were tested.

Hydrolysate clarification

In the analysis of both total glucan and α-glucan content, effective clarification of the sample is a critical step to help ensure accurate and reproducible results. Various clarification methods can be employed depending on the nature of the sample and the available laboratory resources. Among the most commonly used techniques are paper filtration, syringe filtration, and centrifugation.

Sample	Clarification Method	Total Glucan, % *(w/w)*	%CV	α-Glucan, % *(w/w)*	%CV
Cordyceps Militaris	Centrifugation	36.78	0.47	25.05	0.55
	Paper Filtration	37.13		24.97	
	Syringe Filtration	36.93		24.97	
Kit Control (Baker's Yeast Type II)	Centrifugation	25.49	0.11	16.19	0.15
	Paper Filtration	25.45		16.15	
	Syringe Filtration	25.42		16.19	

All three clarification techniques are compatible with both total glucan and α-glucan methodologies as no differences in results were observed across the three methods (i.e. all % CV are below 1 % when comparing to the method results). This allows flexibility in method use and adaptation to different sample types or throughput requirements. The choice of clarification method may depend on practical considerations such as time constraints and equipment availability.

Impact of sample colour

The objective of this study was to evaluate the necessity of incorporating sample blanks in the analytical procedures for Total Glucan and α-Glucan quantification. The study aimed to investigate the potential impact of sample coloration on analytical outcomes across a large dataset.

Method	Degrees of Freedom	t Stat	t Critical two-tail	p value
Total Glucan	42	0.0001	1.989	0.999
α-Glucan	42	0.002	1.989	0.998

The statistical analyses conducted for both Total and α-Glucan methodologies indicate that the inclusion of sample blanks does not significantly influence the results. Coloured samples did not impact the accuracy or variability of Total Glucan results, indicating that sample blanks are unnecessary for this method under the conditions tested. For the α-Glucan assay, highly coloured samples that alter GOPOD colour development from pink to yellow can affect result accuracy, and therefore, the use of sample blanks is recommended in such cases. This is due to the fact that the less sample dilution occurs in the α-glucan methodology.

Overnight Storage of hydrolysates

The aim of this study was to determine whether hydrolysates can be stored overnight at 4°C prior to performing the GOPOD (Glucose Oxidase/Peroxidase) assay. The goal was to assess the feasibility of introducing a pause point in the Total and α-Glucan analytical workflows without compromising data integrity.

Method	Degrees of Freedom	t Stat	t Critical two-tail	p value
Total Glucan	42	2.35	2.02	0.02
α-Glucan	42	0.02	2.02	0.98

The data indicates that while α-Glucan measurements remain stable following overnight storage of hydrolysates (hydrolysates left overnight from Step 6 in Method B) at 4°C, Total Glucan results exhibit a statistically significant shift when hydrolysates are left overnight before testing (hydrolysates left overnight from Step 8 in Method A). As such, the implementation of a pause point is method-dependent: it is acceptable for α-Glucan analysis but not recommended for Total Glucan.

MATRIX INTERFERENCE

Finished mushroom- and yeast-derived products are now available in a diverse range of forms, driven by growing consumer demand and the potential health benefits of these ingredients. However, the presence of potential interfering substances, variation in product matrices, and differences in species composition can impact the accuracy and reliability of analytical results. The objective was to assess matrix interference and the method performance across different sample types. The acceptance criteria for β-glucan spiking recovery was set at ± 5 %, with a coefficient of variation (%CV) below 5 %.

	Total Glucan		α-Glucan	
Sample Type	Spike Recovery, %	%CV	Spike Recovery, %	%CV
Algae product	98.92	0.89	103.03	9.18
Cordyceps Militarius	98.72	1.62	98.36	0.00
Mushroom Capsule	99.13	1.68	98.12	1.75
Mushroom Gummy	97.77	1.70	98.12	1.75
Mushroom Tincture	99.51	0.66	97.83	0.24

All samples demonstrated spike recovery values within the acceptable range (typically 95 – 105 %), indicating high analytical accuracy for both Total and α-Glucan. Most %CV values were below 2 %, showing excellent method precision. The only exception was the α-glucan in the algae product, which showed a %CV of 9.18 %. This elevated variability is attributed to the measured concentration being below the Limit of precision for α-glucan in this matrix.

METHOD COMPARISON

The β-Glucan Assay Kit was updated in October 2025 to include a number of method modifications.

These modifications include:

- Expanded Sample Compatibility – Inclusion of protocols for liquid samples.
- Reduced Reaction Time – Condensed from 7 hours to 4 hours and 25 minutes.
- Optimization of Total Glucan method – Modification to acid hydrolysis procedure and inclusion of an

Hydrolysis Correction Factor (HCF) in to account for glucose loss during acid treatment step.

- Optimization of α-Glucan method - EnzyAlpha™ reagent added, designed to improve α-Glucan hydrolysis in complex matrices.

		β-Glucan, % (*w/w* or *w/v*) *as is*	
		Previous method	New Method (2025)
Pure Mushrooms	*Hericium erinaceus* (Lion's Mane)	42.60	43.57
	Ganoderma lucidum (Reishi)	46.81	50.74
	Inonotus obliquus (Chaga)	12.69	16.83
	Trametes versicolor (Turkey Tail)	52.40	57.53
	Phellinus linteus (Mesima)	20.54	25.08
	Pleurotus ostreatus (Oyster)	25.29	26.33
	Poria cocos	75.58	70.85
	Tremella fuciformis	11.77	10.3
	Pleurotus citrinopileatus (Golden Oyster)	25.73	28.46
	Lentinula edodes (Shiitake)	21.86	24.3
	Grifola frondosa (Maitake)	23.95	32.4
	Cordyceps Sinensis	12.88	26.58
	Cordyceps Militarius	10.22	11.01
Mushroom extracts	Chaga extract 1:1	12.04	13.9
	Chaga extract 8:1	7.28	8.89
	Cordyceps mushroom extract 1:1	10.94	12.9
	Cordyceps mushroom extract 8:1	2.13	3.13
	Poria sclerotia extract 1:1	75.16	81.5
	Shiitake mushroom extract 1:1	26.05	29.02
	Tremella mushroom extract 8:1	8.22	9.39
	Turkey Tail mushroom extract 1:1	55.12	60.93
Mushroom Capsules	Sample 1	4.91	7.23
	Sample 2	-0.77	2.28
	Sample 3	19.38	23.83
	Sample 4	28.59	30.67
	Sample 5	4.29	6.97
	Sample 6	41.29	42.8
	Sample 7	-2.62	1.41
Mushroom Gummies*	Sample 1	26.36	4.24
	Sample 2	3.16	3.92
	Sample 3	-0.76	2.58
	Sample 4	2.76	5.73
	Sample 5	-2.21	2.11
Mushroom Tinctures	Sample 1	0.20	0.19
	Sample 2	6.39	8.46
	Sample 3	4.30	6.90

**The mushroom gummy was analysed using the optional application method outlined in the product instructions.*

		β-Glucan, % (*w/w* or *w/v*) *as is*	
		Previous method	K-YBGL (2025)
Whole cell yeast	Brewer's Yeast	10.06	10.44
	Baker's Yeast	6.07	8.47
Yeast Products	Sample 1	21.81	23.46
	Sample 2	25.81	28.3
	Sample 3	30.72	22.51
	Sample 4	50.95	53.14
	Sample 5	77.43	82.9
	Sample 6	66.95	71.77
Algae Dietary Ingredients (*Euglena*)	Sample 1	50.29	51.92
	Sample 2	80.02	90.81

Results obtained comparing the two methods are shown for a large and varied sample set, including pure mushroom samples, mushroom-based capsules, mushroom tinctures, yeasts and algae products. For most samples, higher β-Glucan values are achieved using the new method.

SERVICES AND TECHNICAL SUPPORT

Please reach out to your local sales representative should you require any assistance, particularly in relation to:

- Troubleshooting
- Data analysis
- Additional matrix testing

Supporting documents can be found in the product page:

- Product Instructions
- Mega-Calc™
- Safety data sheets (SDS)
- Certificate of analysis (CoA)

Contact us for more information: neogen.com/contact

Without guarantee

The information contained in this assay protocol is, to the best of our knowledge, true and accurate, but since the conditions of use are beyond our control, no warranty is given or is implied in respect of any recommendation or suggestions which may be made or that any use will not infringe any patents.

User Responsibility:

- Users are responsible for familiarizing themselves with product instructions and information. Visit our website at neogen.com, or contact your local Neogen® representative or authorized distributor for more information.
- When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, laboratory technique and the sample itself may influence results.
- It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples with the appropriate matrices and challenges to satisfy the user that the chosen test method meets the user's criteria.
- It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.
- As with any test method, results obtained do not constitute a guarantee of the quality of the matrices or processes tested.

Terms and Conditions:

Neogen's full terms and conditions are available online.