



## Validation Report: $\beta$ -Glucan Assay Kit (Yeast & Mushroom) (cat. no. K-YBGL)

### 1. Scope

Megazyme's  $\beta$ -Glucan Assay Kit (Yeast & Mushroom), (K-YBGL), is a colorimetric method used for the measurement and analysis of 1,3:1,6- $\beta$ -D-glucan (plus 1,3- $\beta$ -glucan, if present) and 1,4:1,6- $\alpha$ -glucan (starch/glycogen) in yeast and mushrooms. This method is a novel method developed in-house and measures Yeast  $\beta$ -Glucan in g/100g on an "as is" basis.

### 2. Planning

The purpose of this report is to verify and validate the current method as detailed by the  $\beta$ -Glucan Assay Kit (Yeast & Mushroom) (K-YBGL).

### 3. Performance characteristics

The selectivity, working range, limit of detection, trueness (*bias*) and precision of this kit is detailed in this report.

#### 3.1 Selectivity

This assay measures 1,3:1,6- $\beta$ -D-glucan and 1,3- $\beta$ -D-glucans, both of which occur in mushroom and yeast cell-walls. The method also measures 1,3:1,4- $\beta$ -D-glucan (cereal  $\beta$ -glucan) which is not produced by yeast or fungi and only occurs in yeast or mushroom products if specifically added or if mushroom mycelia is grown on grain such as barley or oats (mycelial products).

This method is not applicable for the specific analysis of mushroom or yeast  $\beta$ -glucan in the presence of cellulose (1,4- $\beta$ -D-glucan) or cereal  $\beta$ -glucans (1,3:1,4- $\beta$ -glucan). In the acid hydrolysis step, cereal  $\beta$ -glucans are completely hydrolysed and cellulose is partially hydrolysed, leading to overestimation of the mushroom or yeast  $\beta$ -glucan.



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### **3.2 Working Range**

The working range for this kit is determined by the D-glucose control provided in the kit.

The glucose measurement (incubation with GOPOD Reagent) is linear between 4 and 100 micrograms of glucose per assay.

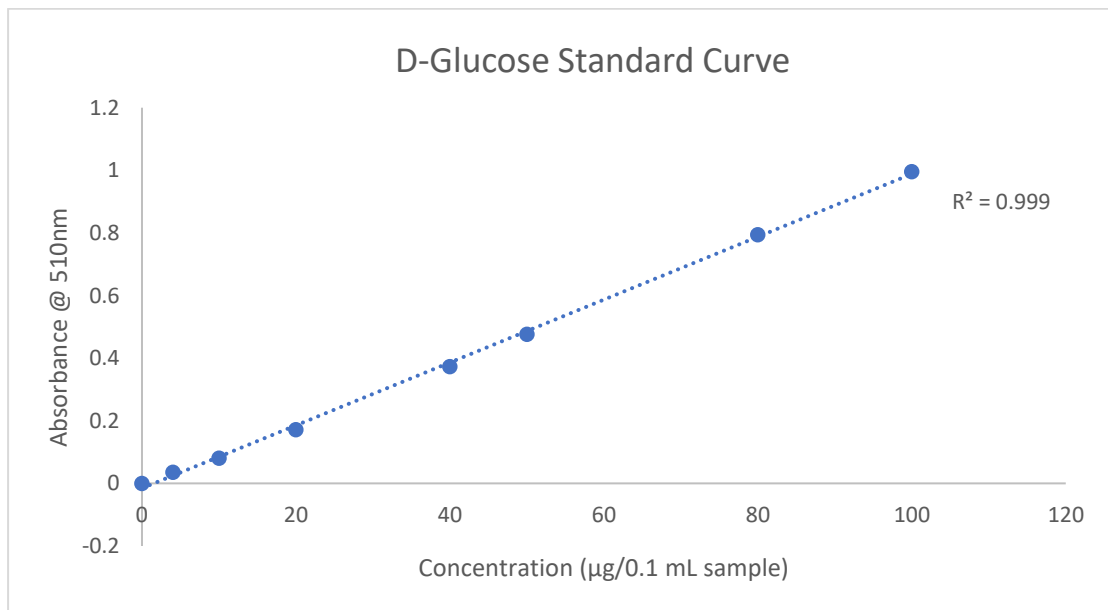
0.1 mL of D-glucose standards at various concentrations is incubated with 3 mL of GOPOD Reagent for 20 min at 40°C. The absorbance values of all samples and blanks read against the reagent blank at 510 nm, as specified in the kit data booklet.

The expected absorbance for 100 µg of the D-glucose control is ~1.1. If the absorbance of any sample is higher than that of 100 µg of D-glucose standard (i.e. higher than 1.1) then the sample must be diluted accordingly.



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D-Glucose Standard Concentration [ $\mu\text{g}/0.1 \text{ mL}$ ]	$\Delta A_{510\text{nm}}$
0	0
4	0.03545
10	0.08105
20	0.17165
40	0.3731
50	0.4768
80	0.79435
100	0.9957





### 3.3 LOD

If the standard procedure is followed, the smallest differentiating recommended absorbance change ( $\Delta A$ ) is 0.04 (equivalent to  $\sim 40 \mu\text{g}$  of D-glucose/mL of sample). This is equivalent to  $\sim 3.6 \%$  w/w of  $\beta$ -glucan in the sample, assuming there is no  $\alpha$ -glucan present. The highest  $\Delta A$  should be lower than the absorbance values obtained for  $100 \mu\text{g}$  of the D-glucose standard. This is equivalent to  $\sim 90 \%$  w/w of  $\beta$ -glucan, assuming there is no  $\alpha$ -glucan present. If the expected  $\beta$ -glucan is higher the sample should be diluted 2-fold with water, prior to incubation with GOPOD Reagent.

\* Note: The above detection limits are for samples as used in the assay, after any sample preparations (e.g. deproteinization). The dilution used in pre-treatment must be accounted for while establishing the detection limits for specific samples.

### 3.4 Trueness (*Bias*)

Comparison of the mean of the results ( $x$ ) achieved with the  $\beta$ -Glucan Assay Kit (Yeast & Mushroom) (K-YBGL) method with a suitable reference value ( $x_{\text{ref}}$ ). For this report, Relative Bias is calculated in per cent as:

$b(\%) = x - x_{\text{ref}} / x_{\text{ref}} \times 100$ . The reference material for this purpose is a yeast  $\beta$ -glucan preparation that is supplied with the  $\beta$ -Glucan Assay Kit (Yeast & Mushroom) (K-YBGL), at  $\sim 49\%$   $\beta$ -glucan content.

#### Relative Bias $b(\%)$

	n	Ref Material (% w/w)	Mean (% w/w)	$b(\%)$
$\beta$ -Glucan	23	49	49.7429	1.52

### 3.5 Precision

This report details the reproducibility of the  $\beta$ -Glucan Assay Kit (Yeast & Mushroom) (K-YBGL), it is a measure of the variability in results, on different days and by different analysts, over an extended period of time.

For the purpose of this report different lot numbers of the kit standard is used as the reference material.



#### Reproducibility

	n	Ref Material (%w/w)	Mean (%w/w)	Standard Deviation	%CV
<b>β-Glucan</b>	23	49	49.7429	0.6530	1.31

#### 4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of β-glucan in yeast and mushroom matrices and has been used for many years. Data presented in this report verifies and validates that this method is fit for the purpose intended and is summarised below.

Validation Summary	Glucose
Working range (µg in assay)	4-100
LOD (ΔA)	0.04
Relative Bias <i>b</i> (%)	1.52
Reproducibility (%CV using yeast β-glucan)	1.31