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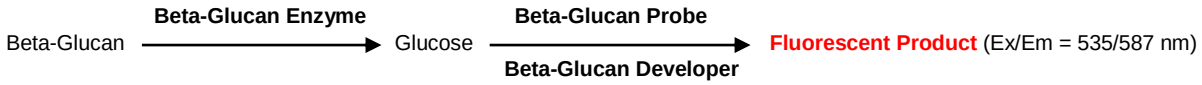
Beta-Glucan Assay Kit (Fluorometric)

12/20

(Catalog # K2068-100; 100 assays; Store at -20 °C)

I. Introduction:

Beta-glucan comprises a group of beta-D-glucose polysaccharides naturally present in the cell walls of grains, bacteria, and fungi. It is a member of the glucan family and a polysaccharide derived from D-glucose, connected by glycosidic bonds. Beta-glucans form a linear backbone with (1-3)-beta-glycosidic bonds, but they vary in molecular mass, solubility, viscosity, branching structure, etc. The presence of (1, 3)-beta-glucan is marketed as a means of identifying invasive or disseminated fungal infections. **BioVision's Beta-Glucan Assay Kit** provides a facile, rapid way to monitor the amount of (1, 3)-beta-glucan in various biological samples. In this kit, (1, 3)-beta-glycosidic bonds are cleaved by an enzyme mix to generate glucose. Glucose then reacts with the Beta-Glucan probe and the developer to generate a fluorescent product measured at Ex/Em = 535/587 nm. The assay is simple, sensitive, and can detect as low as 25 ng of (1, 3)-beta-glucan.



II. Application:

- Measurement of (1, 3)-beta-glucan in various samples.

III. Sample Types:

- Fungus: Yeast, mushrooms, etc.

IV. Kit Contents:

Components	K2068-100	Cap Code	Part Number
Beta-Glucan Assay Buffer	25 ml	NM	K2068-100-1
Beta-Glucan Development Buffer	25 ml	WM	K2068-100-2
Beta-Glucan Enzyme	1 vial	Blue	K2068-100-3
Beta-Glucan Probe	220 µl	Red	K2068-100-4
Beta-Glucan Developer	1 vial	Green	K2068-100-5
Beta-Glucan Standard	80 µl	Yellow	K2068-100-6

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (plate reader)
- Dry bath/block heater or water bath system
- Dounce Tissue Homogenizer (BioVision Cat. # 1998)
- 90% Ethanol
- 6N KOH
- 6N HCl

VI. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay. Upon opening, use within two months.

- **Beta-Glucan Assay Buffer & Beta-Glucan Development Buffer:** Store at 4 °C or -20 °C. Bring to room temperature (RT) before use.
- **Beta-Glucan Enzyme:** Reconstitute the vial in 220 µl **Beta-Glucan Assay Buffer**. Divide into aliquots and store at -20 °C. Keep on ice while in use. Avoid multiple free-thaw cycles.
- **Beta-Glucan Probe:** Thaw at RT before use. Store at -20 °C, protected from light.
- **Beta-Glucan Developer:** Reconstitute the vial in 220 µl **Beta-Glucan Development Buffer**. Divide into aliquots and store at -20 °C. Keep on ice while in use. Avoid multiple free-thaw cycles.
- **Beta-Glucan Standard:** Thaw at RT. Store at -20 °C.

VII. Beta-Glucan Assay Protocol:

1. **Sample Preparation:** Most samples will need pre-treatment to remove free glucose. Weigh the samples (5-10 mg for yeast, 50-100 mg for mushrooms) and homogenize in 1 ml 90% ethanol using a Dounce Tissue Homogenizer (BioVision Cat. # 1998). Additional sonication might be needed for disrupting the cell walls. Warm to 60 °C for 5 min and vortex occasionally. Centrifuge at 10,000 x *g* and RT for 2 min. Decant the supernatant and add 1 ml 90% ethanol. Repeat the wash twice. Extract the washed sample with 0.5 ml 6N KOH and heating on boiling water bath or a block heater for 5 min. Neutralize the sample slowly with 0.5 ml 6N HCl. Centrifuge at 10,000 x *g* and RT for 2 min and collect the supernatant. Dilute the supernatant 5-10 fold in Beta-Glucan Assay Buffer. Add 2-10 µl of the diluted samples into a 96-well clear plate designated as Sample(s) and Sample Background Control. Adjust the volume of **Sample(s)** and **Sample Background Control** wells with Beta-Glucan Assay Buffer to **40 µl/well** and **50 µl/well** respectively.

   Note: We suggest running several dilutions of the Samples to ensure the readings are within the Standard Curve range.

2. **Standard Curve Preparation:** Prepare a 50 µg/ml Beta-Glucan Standard by adding 5 µl of Beta-Glucan Standard stock to 95 µl Beta-Glucan Assay Buffer. Add 0, 2, 4, 6, 8, 10 µl of 50 µg/ml Beta-Glucan Standard into a series of wells to generate 0, 0.1, 0.2, 0.3, 0.4, 0.5 µg/well of Beta-Glucan Standard respectively. Adjust the volume to 40 µl/well with Beta-Glucan Assay Buffer.
3. **Sample Hydrolysis:** Prepare sufficient volume of 5-fold dilution the Beta-Glucan Enzyme (i.e dilute 20 µl of Beta-Glucan Enzyme stock with 80 µl of Beta-Glucan Assay Buffer). Add 10 µl of diluted Beta-Glucan Enzyme to each well containing **Sample(s)** and **Beta-Glucan Standards** and mix well. Incubate at 37 °C for 30 min to hydrolyze the beta-glucan.
4. **Sample Development:** Mix enough reagents for the number of assays to be performed. Prepare 50 µl of Reaction Mix as indicated in the table below:

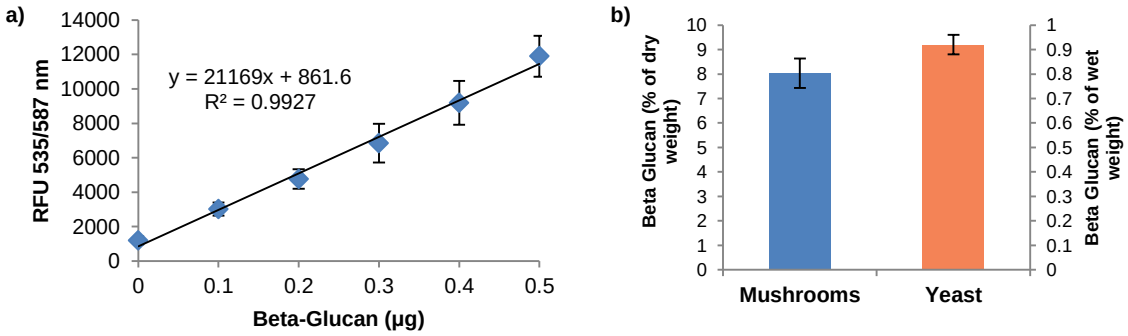
	Reaction Mix
Beta-Glucan Development Buffer	47 µl
Beta-Glucan Probe	1 µl
Beta-Glucan Developer	2 µl

Add 50 µl of Reaction Mix to each wells containing **Sample(s)**, **Sample Background Control** and **Standards**, mix well. The total volume of each well including **Sample(s)**, **Sample Background Control**, and **Standard**, is 100 µl. Mix well and incubate at 37 °C for 30 min, protected from light. **Note:** Prepare Reaction Mix immediately before adding to the wells.

5. **Measurement:** Measure the fluorescence intensity of all wells at 37 °C (Ex/Em = 535/587 nm) in end-point mode.
6. **Calculation:** Subtract 0 Standard reading from all Standard readings. Plot the Beta-Glucan Standard Curve. Subtract the Sample Background Control reading from all Sample readings to get the corrected Sample readings (ΔRFU). Apply the corrected Sample readings (ΔRFU) to Beta-Glucan Standard Curve to obtain the corresponding amount of beta-glucan **(B in µg)** and calculate the percentage of Beta-glucan in the Sample as:

Percentage of Beta-Glucan in the Sample = B x D / (V x P) x 100 %

Where: **B** = Amount of Beta-glucan from the Standard Curve (µg)
V = Sample volume added into the reaction well (µl)
P = Initial Sample weight (mg)
D = Sample dilution factor (D = 1 for undiluted samples)



Figures: (a). Beta-Glucan Standard Curve (results from multiple experiments). **(b).** Percentage of Beta-glucan in mushrooms (50 µg) and yeast (5 µg). All assays were performed following the kit protocol.

VIII. Related Products:

Starch Colorimetric/Fluorometric Assay Kit (K647)
Glucose Colorimetric/Fluorometric Assay Kit (K606)
Glucosylceramidase Activity Assay Kit (Fluorometric) (K2003)
Total Cholesterol and Cholesteryl Ester Colorimetric/Fluorometric Assay Kit (K603)
Dounce Tissue Homogenizer (1998)
Glycogen Colorimetric/Fluorometric Assay Kit (K646)

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