ab303728 – Beta-Glucan Assay Kit (Fluorometric)

For the quantitative measurement of beta-glucan in various biological samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
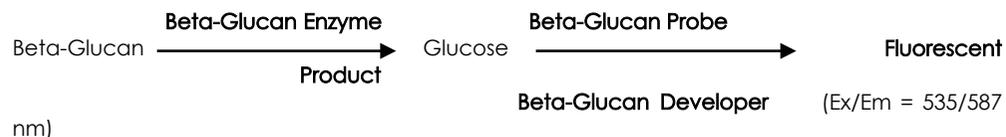http://www.abcam.com/ab303728

Storage and Stability
Store the kit at -20 °C for up to 12 months, protected from light.

Introduction:

The 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.

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.

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                  Beta-Glucan Enzyme                Beta-Glucan Probe
Beta-Glucan ─────────────────────────▶ Glucose ─────────────────────────▶ Fluorescent
                       Product                     Beta-Glucan Developer        (Ex/Em = 535/587 nm)
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Materials Supplied

Components	
Beta-Glucan Assay Buffer	25 ml
Beta-Glucan Development Buffer	25 ml
Beta-Glucan Enzyme	1 vial
Beta-Glucan Probe	220 µl
Beta-Glucan Developer	1 vial
Beta-Glucan Standard	80 µl

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

– 96-well clear plate with flat bottom
– Multi-well spectrophotometer (plate reader)
– Dry bath/block heater or water bath system
– Dounce Tissue Homogenizer
– 90% Ethanol

– 6N KOH
– 6N HCl

Reagent Preparation

– Briefly centrifuge all small vials at a low speed prior to opening
– Read the entire protocol before performing the experiment.

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.

Standard Preparation

1. Prepare a 50 µg/ml Beta-Glucan Standard by adding 5 µl of Beta-Glucan Standard stock to 95 µl Beta-Glucan Assay Buffer.
2. 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.

Sample Preparation

– Most samples will need pre-treatment to remove free glucose.

1. 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. 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.
2. 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.
3. Dilute the supernatant 5-10 fold in Beta-Glucan Assay Buffer.
4. 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.

Assay Procedure

– It is recommended that all standards and samples be run at least in duplicate.
– Prepare all reagents, Standards and sample(s) as instructed
– Prepare enough reagents for the number of assays to be performed
– A standard curve should be run for each assay.

Sample Hydrolysis:

1. 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).
2. 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.

Sample Development:

1. 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

2. 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.

Measurement
Measure the fluorescence intensity of all wells at 37 °C (Ex/Em = 535/587 nm) in end-point mode.

Calculations

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)

For technical support contact information, visit: www.abcam.com/contactus