Mannanase Assay Kit

ARG83398 Mannanase Assay Kit is a detection kit for the quantification of Mannanase in Tissue extracts, cell lysate and other biological fluids.

Catalog number: ARG83398

Package: 96 wells

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INTRODUCTION

β-Mannanases (endo-1,4-β-d-mannanase) is endohydrolase that catalyze the random hydrolysis of the β-1,4-d-mannopyranosyl linkage within the main chain of various mannan-based polysaccharides to yield mannooligosaccharides products. β-Mannanase have been isolated and characterized from different sources including bacteria, fungi, higher plants, and animals. However, microbial mannanases are wildly used in the industrial application. β-Mannanases was classified based on the amino acid sequence similarity into glycoside hydrolase (GH) families 5 and 26 and a few member of family 113.

PRINCIPLE OF THE ASSAY

ARG83398 Mannanase Assay Kit determined endo-beta-Mannanase hydrolyzes the mannan to generate mannose. Mannose react with 3,5-dinitrosalicylic acid to generate red-brown substance. The intensity of the color is measured at a wavelength of 540 nm. The concentration of Mannanase in the sample is determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the Positive Control at-20°C, all other at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4°C
Positive Control	1 vial (lyophilized)	-20°C
Substrate	1 vial (lyophilized)	4°C
Reagent Dye	10 ml	4°C
Assay Buffer	30 ml x 4	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 540nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the Positive Control at-20°C, all other at 2-8°C. Use the kit before expiration date.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

For Tissue- Weigh out 0.1 g tissue, homogenize with **1 ml** of **Assay buffer** on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Cell and bacteria- Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add **1 ml** of **Assay buffer** for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

REAGENT PREPARATION

- **Positive Control:** Reconstitute the **Positive Control** with **0.1 ml** of **Assay buffer**.
- **Substrate:** Reconstitute the **Substrate** with **8 ml** of **Assay buffer**.
- **Standard:** Reconstitute the **Standard** with **1 ml** of **distilled water** to yield **10 µmol/mL** Standard. Perform 2-fold serial dilution of the top standard (10 µmol/mL) to make the standard curve.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample: Add **20 µl** of **Samples** into Sample well.
2. Control: Add **20 µl** of **Assay Buffer** into Control well.
3. Positive Control: Add **20 µl** of **Positive Control** into Positive Control well.
4. Add **80 µl** of **Substrate** into Sample, Control and Positive Control well.
5. Mix well. Incubate at **37°C** oven for **10 min**.
6. Standard: Add **100 µl** of **Standard** into Standard well.
7. Blank: Add **100 µl** of **distilled water** into Blank well.
8. Add **100 µl** of **Substrate** into each well.
9. Mix well. Incubate at **90°C** for **10min**. Read the OD at **540nm**.

Summary of Mannanase Assay Kit Procedure

Reagent	Sample	Control	Positive Control	Standard	Blank
Sample	20 µl	-	-	-	-
Assay Buffer	-	20 µl	-	-	-
Positive Control	-	-	20 µl	-	-
Substrate	80 µl	80 µl	80 µl	-	-
Mix well. Incubate at **37°C** oven for **10 min**.					
Standard	-	-	-	100 µl	-
distilled water	-	-	-	-	100 µl
Reagent Dye	100 µl	100 µl	100 µl	100 µl	100 µl
Mix well. Incubate at **90°C** oven for **10 min**.					
Read the OD with a microplate reader at **540 nm**.					

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, control and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

7. Calculation:

A. Unit Definition: One unit of endo-beta-Mannanase activity is the enzyme generates 1 μmol of mannose per minute

$C_{Standard}$: the standard concentration, 10 μmol/mL;

$C_{Protein}$: the protein concentration, mg/mL;

V_{Assay}: the volume assay buffer, 1 mL;

V_{Sample}: the volume of reaction sample, 0.02 mL;

$V_{standard}$: the volume of standard sample, 0.1 mL;

W: the weight of sample, g;

T: the reaction time, 10 minutes.

B. Formula:

I. According to the concentration:

Mannanase (U/mg) = $[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{Control})] / [(OD_{Standard} - OD_{Blank}) \times ((V_{Sample} \times C_{Protein}) \times T]$

= $\mathbf{5 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times C_{Protein}]}$

II. According to the weight:

Mannanase (U/g) = $[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{Control})] / [(OD_{Standard} - OD_{Blank}) \times (W \times V_{Sample} / V_{Assay}) \times T]$

= $\mathbf{5 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times W]}$

III. According to the quantity of cell or bacteria:

Mannanase ($U/10^4$) = $[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{Control})] / [(OD_{Standard} - OD_{Blank}) \times (N \times V_{Sample} / V_{Assay}) \times T]$

= $\mathbf{5 \times (OD_{Sample} - OD_{Control}) / [N \times (OD_{Standard} - OD_{Blank})]}$

8. Detection range:

The detection range is from 1 μmol/mL- 10 μmol/mL.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and serial diluted standards are not necessary for this kit.