Mannanase Activity Assay Kit

ARG83398 Mannanase Assay Activity Kit can be used to measure Mannanase in Tissue extracts, cell lysate and other biological fluids.

Catalog number: ARG83398

Package: 96 wells

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INTRODUCTION

endo-beta -mannanases have been used in a wide range of industries such as feed, detergent, biorefinery and textile. The production and use of β-mannanases are on the rise due to increased awareness of their utility and the incorporation of enzyme engineering and gene manipulation techniques.

PRINCIPLE OF THE ASSAY

The ARG83398 Mannanase Activity Assay Kit determined Mannanase by Mannanase hydrolyzes the mannan to generate mannose. Mannose react with 3,5-dinitrosalicylic acid to generate red-brown substance. The increase in absorbance at 540 nm is directly proportional to the enzyme activity.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit 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
Reaction Dye	10 ml	4°C
Assay Buffer	4 X 30 ml	4°C
Positive Control	1 vial (lyophilized)	-20°C
Substrate	1 vial (lyophilized)	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 Positive Control at -20°C, other component at 4°C.
- Reaction Dye should be store at 4°C and protect from light.
- 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 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.

For cell and bacteria- Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5 × 106 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

- **Standard:** Add **1 ml** of **distilled water** to yield **10 µmol/ml** standard. Perform 2-fold serial dilution of the top standards to make the standard curve.
- **Substrate:** Reconstitute the Substrate with **8 ml** of **Substrate Diluent**. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.
- **Positive Control:** Dilute the Positive Control with **100 µl** of **Assay Buffer.** Make sure the Positive Control is mixed thoroughly before use. Keep the Positive Control on ice before use.
- **Sample**: If the measuring absorbance of samples is higher than the standard, dilute the samples with **assay buffer** before assay and assay again.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

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

Summary of Mannanase 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 all Sample tubes at 37°C oven for 10 min.					
Standard	-	-	-	100 µl	-
Distilled water	-	-	-	-	100 µl
Reaction 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. Unit Definition: One unit activity is defined as the enzyme that hydrolysis of one µmol arginine per minute.

2. Calculate the average absorbance values for each set of samples, standard, positive control, control and blank.

3. Calculation:

A. Definition:

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

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

W: the weight of sample, g;

V_{Sample}: the volume of reaction sample, 10 μl = 0.01 ml;

$V_{standard}$: the volume of standard sample, 10 μl = 0.02 ml;

T: the reaction time, 10 minutes.

B. Formula:

a). According to the protein concentration of sample

Mannanase activity (U/mg) =

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

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

b). According to the weight of sample

Mannanase activity (U/g) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample}-OD_{Control})] / [(OD_{Standard}- OD_{Blank}) \times W \times (V_{Sample} / V_{total}) \times T]$

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

c). According to the quantity of cells or bacteria

Mannanase activity ($U/10^4$) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample}-OD_{Control})] / [(OD_{Standard}- OD_{Blank}) \times N \times (V_{Sample} / V_{total}) \times T]$

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

4 Detection range:

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

5. If the samples have been diluted, the calculated concentration must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

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.