Amylose Assay Kit

ARG83394 Amylose Assay Kit can be used to measure Amylose in Tissue extracts and other biological fluids.

Catalog number: ARG83394

Package: 96 wells

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Tel: +886-3-6221320

Fax: +886-3-5530266

Email: info@arigobio.com

INTRODUCTION

Amylose is a polysaccharide made of α-D-glucose units, bonded to each other through α(1⟶4) glycosidic bonds. It is one of the two components of starch, making up approximately 20–30%. Because of its tightly packed helical structure, amylose is more resistant to digestion than other starch molecules and is therefore an important form of resistant starch.

PRINCIPLE OF THE ASSAY

The ARG83394 Amylose Assay Kit determined Amylose by the pure blue in various samples. The increase in absorbance at 630 nm is directly proportional to the content.

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 (8 mg)	1 vial (lyophilized)	4°C
Assay Buffer A	4 X 30 ml	4°C
Assay Buffer B	4 X 30 ml	4°C
Reaction Buffer A	10 ml	4°C
Reaction Buffer B	8 ml	4°C
Reaction Dye	1 ml	4°C (protect from light)

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 630 nm
- 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 all 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.

Tissue lysate- Weigh 0.01 g tissue, homogenize with 1 ml Assay Buffer A, then transfer all the lysate to the microtube, centrifuged at 4000g for 10 minutes, discards the supernatant; then add 1 ml Assay Buffer B, warm at 80 °C for 10 mins, centrifuged at 4000g for 10 minutes, take the supernatant into a new centrifuge tube for detection.

Note: For other liquid sample, it can be assayed directly.

REAGENT PREPARATION

- **Standard:** Add **1 ml** of **Assay Buffer B** to yield 8 mg/ml standard. Perform 2-fold serial dilution of the top standards to make the standard curve.
- **Sample**: If the measuring absorbance of samples is higher than the standard, dilute the samples with **distilled water** before assay and assay again. For the calculation of the activity this dilution factor has to be taken into account.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Sample wells: Add **10 µl** per **samples** into Sample wells.
2. Standard wells: Add **10 µl** of **Standard** into Standard wells.
3. Add **100 µl Reaction Buffer A** into All wells.
4. Add **80 µl Reaction Buffer B** into All wells.
5. Add **10 µl** of **Reaction Dye** into All wells.
6. Mix well. Incubate at **RT** for **5 min**.
7. Read the OD with a microplate reader at **630 nm**.

Summary of Amylose Assay Kit Procedure

Reagent	Sample	Standard	Blank
Sample	10 µl	-	-
Standard	-	10 µl	-
Distilled water	-	-	10 µl
Reaction Buffer A	100 µl	100 µl	100 µl
Reaction Buffer B	80 µl	80 µl	80 µl
Reaction Dye	10 µl	10 µl	10 µl
Mix well. Incubate at **RT** oven for **5 min**.			
Read the OD with a microplate reader at **630 nm**.			

CALCULATION OF RESULTS

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

2. Calculation:

A. Definition:

$C_{Standard}$: the standard concentration, 8 mmol/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 = 0.01 ml;

V_{assay}: the volume of standard sample, 1000 µl = 1 ml;

B. Formula:

a). According to the volume of sample

Amylose (mg /ml) =

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

$= 8 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

b). According to the concentration of sample

Amylose (mg /g) =

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

$= 8 \times (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) \times W)]$

3 Detection range:

The detection range is from 800 µmol/ml - 8000 µmol/ml.

4. 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 serially diluted standards are necessary for this kit.