Amylopectin Assay Kit

ARG83395 Amylopectin Assay Kit can be used to measure Amylopectin in Tissue extracts and other biological fluids.

Catalog number: ARG83395

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Amylopectin, the highly branched molecule, is usually the major component in the starch granule with α(1–4)-linked glucose linear chains and α(1–6)-linked branch points. Crystalline domains of the starch granules are due to the clustered branches of amylopectin chains that are packed together, whereas the free amylose, amylose complexed with lipids, and branch points of the amylopectin are found in the amorphous region. Alternative arrangement of crystalline and amorphous region was proposed for the semicrystalline starch granule.

PRINCIPLE OF THE ASSAY

The Amylopectin Assay Kit determined Amylopectin by the purplish red in various samples. The measurement wavelength and reference wavelength of the amylose were 550nm and 735 nm. The absorbance difference between the two wavelengths 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	RT
Standard	1 vial (lyophilized)	4°C
Assay Buffer	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 550nm and 735nm
- 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, keep Reaction Dye 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.

<u>Tissue lysate-</u> Weigh 0.01 g tissue, homogenize with 1 ml Assay buffer, then transfer all the lysate to the microtube, centrifuged at 4000g for 10 minutes; take the supernatant into a new centrifuge tube for detection.

REAGENT PREPARATION

- **Standard:** Add **1 ml** of **Assay Buffer** to yield <u>4000 μg/ml</u> 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. <u>Sample wells</u>: Add **10 μl** of **samples** into <u>Sample wells</u>.
2. <u>Standard wells</u>: Add **10 μl** of **Standard** into <u>Standard wells</u>.
3. Add **100 μl Reaction Buffer A** into <u>All wells</u>.
4. Add **80 μl Reaction Buffer B** into <u>All wells</u>.
5. Add **10 μl** of **Reaction Dye** into <u>All wells</u>.
6. Mix well. Incubate at **RT** for **5 min**.
7. Read the OD with a microplate reader at **550nm and 735nm**.

Summary of <u>Amylopectin Assay Kit</u> 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 **550nm and 735nm**.			

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, 4000 µg/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) =

        $\{(C_{Standard} \times V_{standard}) \times [(OD_{Sample550} - OD_{Sample735}) - OD_{Blank}]\} / \{[(OD_{Standard550} - OD_{Standard735}) - OD_{Blank}] \times V_{Sample}]\}$

        $= 4 \times [(OD_{Sample550} - OD_{Sample735}) - OD_{Blank}] / [(OD_{Standard550} - OD_{Standard735}) - OD_{Blank}]$

    b). According to the concentration of sample

        Amylose (mg / g) =

        $\{(C_{Standard} \times V_{standard}) \times [(OD_{Sample550} - OD_{Sample735}) - OD_{Blank}]\} / \{[(OD_{Standard550} - OD_{Standard735}) - OD_{Blank}] \times (V_{Sample} \times W / V_{Assay})\}$

        $= 4 \times [(OD_{Sample550} - OD_{Sample735}) - OD_{Blank}] / \{[(OD_{Standard550} - OD_{Standard735}) - OD_{Blank}] \times W\}$

3 Detection range:

The detection range is from 100 µg/ml- 4000 µg/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 serial diluted standards are not necessary for this kit.