Resistant Starch

Microplate Assay Kit

User Manual

Catalog # CAK1124

(Version 1.3A)

Detection and Quantification of Resistant Starch (RS) Content in Tissue extracts, Food, Other agricultural products Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Resistant starch (RS) is starch, including its degradation products, that escapes from digestion in the small intestine of healthy individuals. Resistant starch occurs naturally in foods but is also added to foods by the addition of isolated or manufactured types of resistant starch. Some types of resistant starch (RS1, RS2 and RS3) are fermented by the large intestinal microbiota, conferring benefits to human health through the production of short-chain fatty acids, increased bacterial mass, and promotion of butyrate-producing bacteria. Resistant starch in various ways has similar physiologic effect as dietary fiber, which is why it functions as a mild laxative and why consuming it at high doses can lead to flatulence.

Resistant Starch Microplate Assay Kit is a sensitive assay for determining resistant starch in various samples. Non-resistant starch is solubilised and hydrolysed to D-glucose by the combined action of the pancreatic α-amylase and amyloglucosidase. The remaining resistant starch is quantitatively hydrolysed to glucose with AMG. D-Glucose is measured with glucose oxidase/peroxidase reagent and this is a measure of the resistant starch content of the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	--
Assay Buffer	20 ml x 1	4 °C
Enzyme A	Powder x 1	-20 °C
Enzyme B	Powder x 1	-20 °C
Enzyme Diluent	30 ml x 1	4 °C
Reaction Buffer	4 ml x 1	4 °C
Dye Reagent A	Powder x 1	-20 °C
Dye Reagent B	Powder x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	--
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Note:

Enzyme A: add 25 ml Enzyme Diluent to dissolve before use, store at 4 °C.

Enzyme B: add 1 ml distilled water to dissolve before use, store at 4 °C.

Dye Reagent A: add 7 ml distilled water to dissolve before use.

Dye Reagent B: add 7 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use, mix, the concentration will be 4 mg/ml.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 505 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Tube revolver
7. Centrifuge
8. Timer
9. Oven
10. Ethanol

IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.01 g tissue, pound in a mortar with 0.25 ml Enzyme A and 0.25 ml distilled water, transfer all reagent into a centrifuge tube; put the tubes into the oven, incubate them at 37°C for 16 hours with tube revolver; add 0.5 ml ethanol with vigorous stirring on a vortex mixer. Centrifuge the tubes at 1,500 g for 10 minutes; Decant the supernatants and re-suspend the pellets in 1 ml of 50% ethanol with vigorous stirring on a vortex mixer, mix the tubes and centrifuge again at 1,500 g for 10 minutes. Decant the supernatants and repeat this suspension and centrifugation step once more. Add 0.2 ml Assay Buffer to each tube and re-suspend by stirring.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	10 µl	10 µl	--
Reaction Buffer	40 µl	--	--
Enzyme B	10 µl	--	--
Distilled water	--	50 µl	60 µl
Mix, put the plate into the oven, 50 °C for 30 minutes.			
Dye Reagent A	70 µl	70 µl	70 µl
Dye Reagent B	70 µl	70 µl	70 µl
Mix, put the plate into the oven, 37 °C for 15 minutes, record absorbance measured at 505 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the weight of sample

$$RS\ (mg/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) \times 20 \times 162 / 180$$

$$= 14.4 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the concentration of Standard, 4 mg/ml;

W: the weight of sample, g;

$V_{Standard}$: the volume of standard, 0.01 ml;

V_{Sample}: the volume of sample, 0.01 ml;

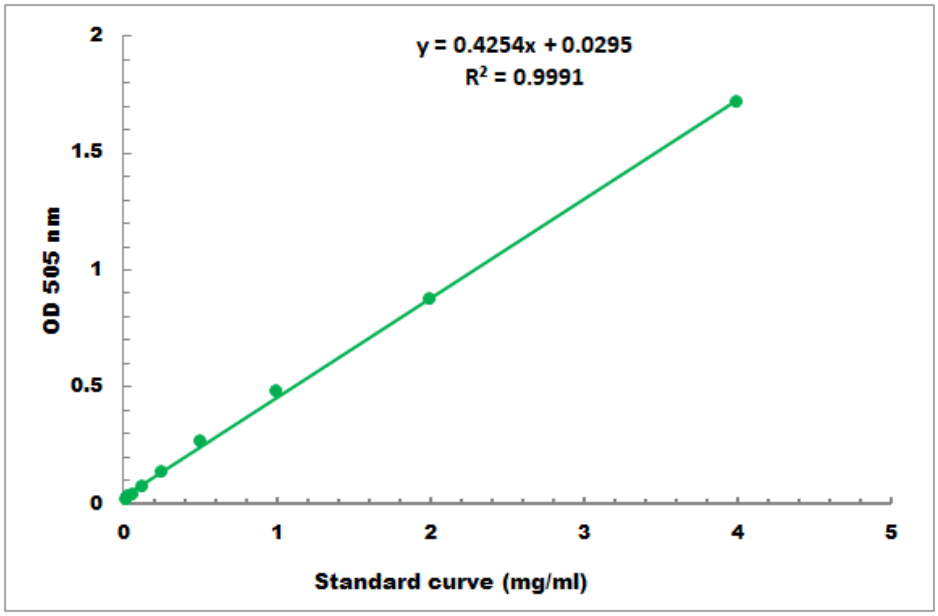
V_{Assay}: the volume of Assay buffer, 0.2 ml;

20: sample volume correction (0.01 ml taken from 0.2 ml);

162 / 180: factor to convert from free D-glucose, as determined, to anhydro-D-glucose as occurs in starch.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.04 mg/ml - 4 mg/ml

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES