Resistant Starch

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1124

(Version 1.4B)

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 StarchColorimetric 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 andis a measure of the resistant starch content of the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	--
Assay Buffer I	Powder x 1	4 °C
Assay BufferII (10X)	20 ml x 2	4 °C
Enzyme I	Powder x 1	-20 °C, keep in dark
Enzyme IDiluent (2X)	25 ml x 4	4 °C
Enzyme II	Powder x 5	-20 °C, keep in dark
EnzymeIII	Powder x 1	-20 °C, keep in dark
Enzyme III Diluent	1 ml x 1	4 °C
Enzyme IV	Powder x 1	-20 °C, keep in dark
Reaction Buffer	17 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	--
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550 nm
2. Distilled water
3. Boiling water bath and 50°C water bath
4. Pipettor, multi-channel pipettor
5. Pipette tips
6. Ice
7. Centrifuge
8. Timer
9. Rotary or Platform Shaker
10. Ethanol
11. 50 % v/v aqueous ethanol

IV. REAGENT PREPARATION

Enzyme I Diluent: Dilute 2X Enzyme I Diluent with an equal volume ofdistilled water to make 1X Enzyme I Diluent (eg. 100 ml to 100 ml2X Enzyme I Diluent).

Enzyme I: Briefly centrifuge prior to opening. Dissolvein200 ml 1X Enzyme I Diluentto makeEnzyme I solution.Divide into small aliquots and store at -20 °C for 6 months.

Enzyme II: Briefly centrifuge prior to opening. Dissolveeach Enzyme II vial in40 ml Enzyme I solution to make Enzyme I&II working solution immediately before use.

Assay Buffer I: Dissolvein100 ml distilled water.Store at 4°C for 2-3 months.

Assay Buffer II: Dilute 10X Assay Buffer II with 9 volumes ofdistilled waterto make 1X Assay Buffer II (eg.20 ml to 180 mldistilled water).

Enzyme III: Briefly centrifuge prior to opening. Dissolvein1ml Enzyme IIIDiluent before use. Store at 4 °C for 7 days or -20°C for 3 months.

Enzyme IV: Briefly centrifuge prior to opening. Dissolvein1ml Reaction Buffer before use. Store at 4 °C for 3-7 days.

Dye Reagent:Add1 ml distilled water into the vial, dissolve it absolutely before use.Store at 4 °C for 3-7 days.

Standard: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use.Then add 0.1 ml into 0.9 ml distilled water, mix, the concentration will be 0.5 mg/ml, store at 4 °C for 2 weeksafter reconstitution.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 0.5/0.25/0.125/0.0625/0.0312/0.0156 mg/ml.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For solid samples

Weigh 50 mg samplesinto a tube. Add 2ml Enzyme I&II working solution and mix by vortex mixer. Put the tubes into the oven, incubate them at 37°C for 16 hours with tube revolver. Add 4 ml ethanol with vigorous stirring on a vortex mixer. Centrifuge the tubes at 1,500 g for 10minutes. Decant the supernatants and re-suspend the pellets in 5 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.Decant the supernatantsand invert the tubes to drain excess liquid. The pellets will be used as follows.

VI. ASSAY PROCEDURE

1.Add following reagents intothe microcentrifuge tubes.

Reagent*	Sample	Positive Control	Blank
Sample	Treated pellet	--	--
Positive Control	--	Provided powder	--
Assay BufferI	1 ml	1 ml	1 ml
Stir the pellets and incubate at 4°C for 20 minwith tube revolver.			
Assay BufferII	4 ml	4 ml	4 ml

2.Mix and transfer 100 µl of product to a new tube, immediately add following reagents.

Reagent*	Sample	Positive Control	Blank
Product	100µl	100 µl	100 µl
Enzyme III	10µl	10µl	10µl
Mix and incubate the tube in a50°C water bath for 30 min. Centrifuge the tube at 3000 rpm for 10 min.			

3.Transfer 20 µl supernatant tothe microplate, then add following reagents into the microplate.

Reagent*	Sample**	Standard	Positive Control	Blank
Reaction Buffer	160 µl	160 µl	160 µl	160 µl
Supernatant	20 µl	--	20 µl	20 µl
Standard	--	20 µl	--	--
Enzyme III	10 µl	10 µl	10 µl	10 µl
Dye Reagent	10 µl	10 µl	10 µl	10 µl
Mix, put in the oven 37 °C for 15minutes, record absorbance measured at 550 nm.				

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.If reading exceeds maximum value of standard curve, we recommend to appropriately diluent the product from the Step 1 and proceed repeatedly according to Step 2. The dilution factor is denoted as n.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-Intercept}{Slope}\times\frac{110}{100}\times 50\times\frac{162}{180}\times n(mg/ml)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1. According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times V_{Sample}}\times\frac{110}{100}\times 50\times\frac{162}{180}\times n\ \ (mg/g)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mg/ml

$V_{Standard}$: the volume of standard in assay procedure, µl

V_{Sample}: the volume of sample in assay procedure, µl

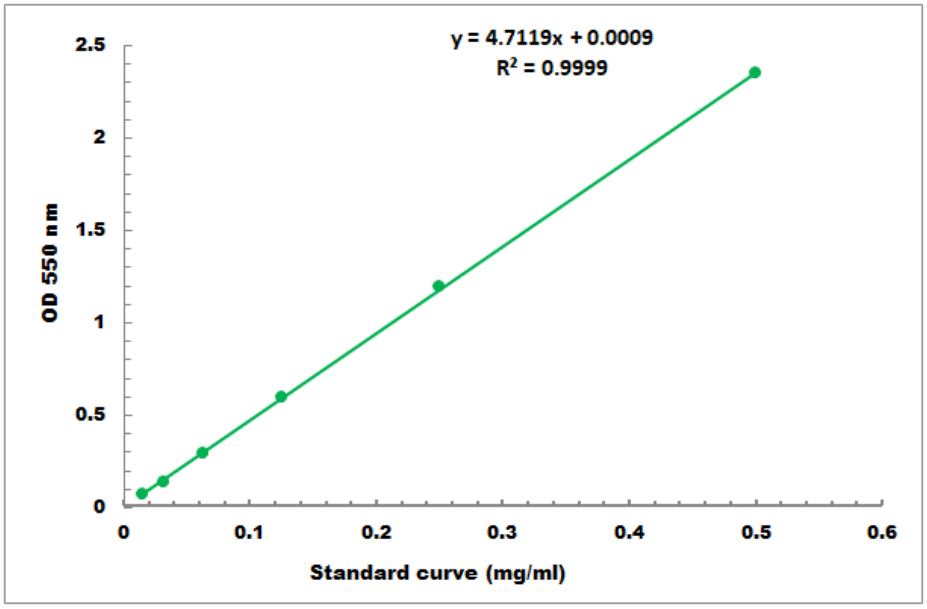
V_{Assay}: the volume of Assay Buffer, µl

W: the weight of sample, g

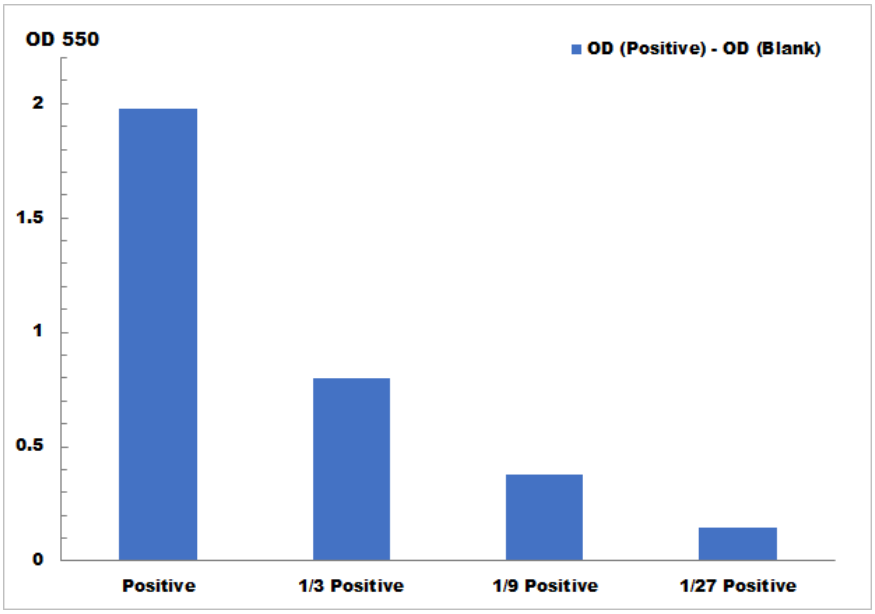
$\frac{162}{180}$:adjustment from free D-glucose to Starch

VII. TYPICAL DATA

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



Detection Range: 0.01 mg/ml-0.5 mg/ml



Resistant starch as positive control reaction with decreasing the concentration