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Validation Report: Resistant Starch Assay Kit (Rapid) (cat. no. K-RAPRS)

1. Scope

Megazyme's Resistant Starch Assay Kit (Rapid) (K-RAPRS), is an enzymatic method used for the measurement and analysis of resistant starch in pure starch, cereal, and food samples. This method is an updated modification of AACC Method 32-40.01, AOAC Method 2002.02 and CODEX Method Type II and measures resistant starch in g/100g on an "as is basis" – if moisture content is known, resistant starch can also be measured in g/100g on a "dry weight basis".

2. Planning

The purpose of this report is to verify and validate the current method as detailed by the Resistant Starch Assay Kit (Rapid) (K-RAPRS).

3. Performance characteristics

The selectivity, working range, limit of detection, trueness (*bias*) and precision of this kit is detailed in this report.

3.1. Selectivity

The assay is specific for D-glucose derived from resistant starch. Non-resistant (digestible) starch values would also include free D-glucose and/or maltodextrins if they are present in the sample.

3.2. Working Range

The working range for this kit is determined by the D-glucose control provided in the kit.
The glucose measurement (incubation with GOPOD Reagent) is linear between 4 and 100 µg of glucose per assay.

0.1 mL of D-glucose standards at various concentrations incubated with 3 mL of GOPOD Reagent for 20 min at 50°C. The absorbances read against the reagent blank at 510 nm, as specified in the kit data booklet.

The absorbance for 100 µg is ~ 1.0. If the absorbance of your samples is higher than that of 100 µg of D-glucose control (i.e. higher than 1.0) they must be diluted accordingly.

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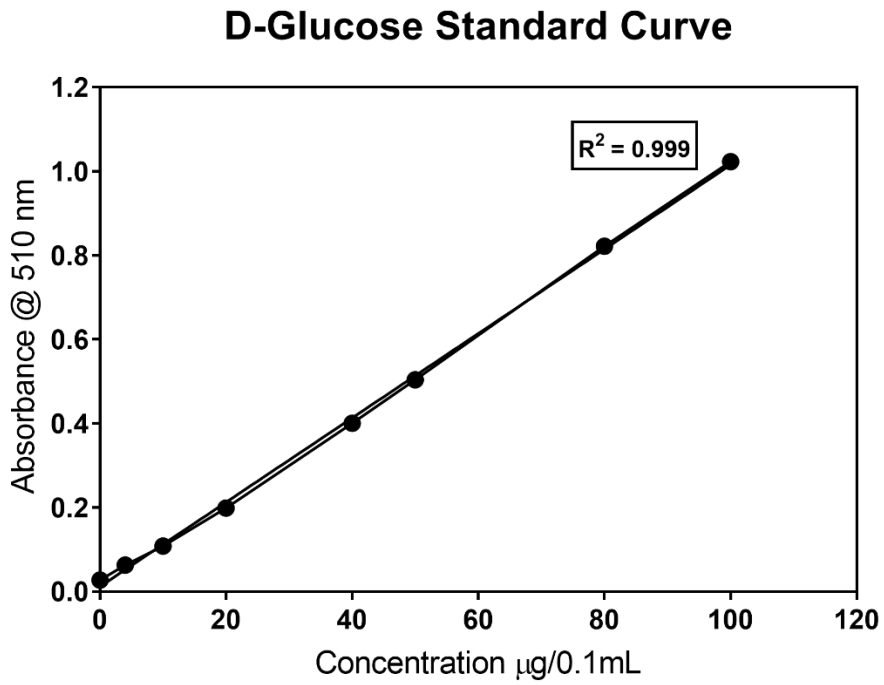

3.3 LOD

If the standard procedure is followed, the smallest differentiating recommended absorbance change (ΔA) is 0.04. This corresponds to ~ 0.35 g/100g resistant starch "as is", using a sample weight of 100 mg and extract volume of 10.3 mL. The highest ΔA should be lower than the absorbance values obtained for 100 µg of glucose. This is equivalent to ~ 9.1 g/100g of resistant starch. If an expected resistant starch content in the sample is > 10% or received absorbance values for the sample are higher than the absorbance values obtained for the glucose standard, the sample solution must be diluted before incubation with GOPOD Reagent.

*** Note:** The above detection limits are for samples as used in the assay, after sample preparations if required (e.g. deproteinisation). The dilution used in pre-treatment must be accounted for while establishing the detection limits for specific samples.

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3.4 Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with the Resistant Starch Assay Kit (Rapid) (K-RAPRS) method with a suitable reference value (x ref). For this report, Relative Bias is calculated in per cent as: ***b(%)* = x – xref / xref x 100**. The reference material for this purpose is the resistant starch control which is supplied with the Resistant Starch Assay Kit (Rapid) (K-RAPRS), at 47.4 g/100g of resistant starch and 35.9 g/100 g digestible starch content.

Relative Bias *b(%)*

	n	Ref Material (g/100g)	Mean (g/100g)	*b*(%)
Resistant Starch	18	47.4	46.46	-1.99
Digestible Starch	18	35.9	35.46	-1.23

3.5 Precision

This report details the reproducibility of the Resistant Starch Assay Kit (Rapid) (K-RAPRS), it is a measure of the variability in results, on different days and by different analysts, over an extended period of time.

For the purpose of this report different lot numbers of the kit standard is used as the reference material.

Reproducibility

	n	Ref Material (g/100g)	Mean (g/100g)	Standard Deviation	%CV
Resistant Starch	18	47.4	46.46	0.2248	0.48
Digestible Starch	18	35.9	35.46	0.4649	1.31

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Repeatability

The repeatability (%RSD_r) of the Rapid Resistant Starch assay method was assessed using 7 milled samples. For each sample, duplicate extractions were processed and applied to the assay on each day across 4 separate days. The resistant starch content of the samples tested covered a working range of 1.8 to 63.9% (w/w). The repeatability (%RSD_r) across this sample data set was extremely high, less than or equal to 7.24% for samples containing 10 to 100% (w/w) resistant starch and less than or equal to 5.31% for samples containing 0.2 to 10% (w/w) resistant starch. This level of repeatability and precision indicates that the Rapid Resistant Starch Assay method is reliable and repeatable, and therefore suited to the application of measuring resistant starch in various food samples including pulses, cereals, fruits and vegetables.

Sample	Resistant Starch, % (w/w)[a], mean[b] ±2 SD, (%RSD_r [c])				
	Day 1	Day 2	Day 3	Day 4	Interday mean, ±2 SD, (%RSD_r)
Regular Maize Starch Lot 60401	1.7 ± 0.2	1.8 ± 0.2	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.2
	5.07	5.09	1.52	3.23	4.33
Hylon VII	48 ± 0.2	48.7 ± 0.4	49.2 ± 0.2	48.6 ± 0.5	48.6 ± 0.9
	0.25	0.45	0.25	0.48	0.92
Tinned Garden Peas	8 ± 0.3	8 ± 1.1	8.5 ± 0.4	8 ± 0.6	8.1 ± 0.6
	1.79	6.74	2.48	3.50	3.94
UB Express Boiled Rice	2.6 ± 0.2	2.3 ± 0.1	2.5 ± 0	2.4 ± 0.2	2.5 ± 0.3
	3.12	1.91	0.89	3.95	5.31
Native Potato Starch Sigma S4251	63.1 ± 0.3	66.2 ± 9.2	68.5 ± 4.2	66 ± 0.2	65.9 ± 5.6
	0.23	6.96	3.03	0.13	4.23
Green Banana	45.6 ± 2.3	46.2 ± 2.1	48.7 ± 2.1	47.1 ± 3.7	46.9 ± 3.2
	2.48	2.32	2.18	3.95	3.44
ActiStar	48 ± 0.5	50.4 ± 0.4	50.5 ± 0.3	48.8 ± 0.2	49.4 ± 2.2
	0.57	0.38	0.28	0.23	2.26

[a] all results are presented as starch on an "as is" basis.

[b] on each day, samples of each material were analysed in duplicate.

[c] SD = standard deviation; RSD_r % = repeatability standard deviation.

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Similarly, the repeatability (%RSD_r) of the Rapid Resistant Starch assay method for the measurement of digestible starch was assessed using 7 milled samples. For each sample, duplicate extractions were processed and applied to the assay on each day across 4 separate days. The digestible starch content of the samples tested covered a working range of 11.5 to 81.6% (w/w). The repeatability (%RSD_r) across this sample data set was extremely high, less than or equal to 9.01%.

Sample	Digestible Starch, % (w/w)[a], mean[b] ± 2 SD, (%RSD_r[c])				
	Day 1	Day 2	Day 3	Day 4	Interday mean, ±2 SD, (%RSD_r)
Regular Maize Starch Lot 60401	80.3 ± 2	81.1 ± 1	82.6 ± 0.5	82.7 ± 1.6	81.6 ± 2.4
	1.25	0.61	0.31	0.99	1.50
Hylon VII	32.2 ± 0.6	33 ± 0.6	33.1 ± 0.1	33.5 ± 1.4	32.9 ± 1.2
	0.93	0.98	0.12	2.08	1.79
Tinned Garden Peas	16.6 ± 0.2	16.6 ± 1.1	17.7 ± 0.5	16.9 ± 0.1	16.9 ± 1.1
	0.53	3.19	1.40	0.28	3.11
UB Express Boiled Rice	69.7 ± 1.2	72.7 ± 0	71.6 ± 0.2	72 ± 1.6	71.5 ± 2.5
	0.88	0.00	0.13	1.09	1.77
Native Potato Starch Sigma S4251	11.4 ± 0.4	11.2 ± 0.2	11.9 ± 0.4	11.3 ± 0.2	11.5 ±0.6
	1.79	0.96	1.51	1.10	2.71
Green Banana	17.3 ± 1.5	17.7 ± 0.2	14.8 ± 1.2	16.2 ± 2.7	16.5 ± 2.7
	4.30	0.63	4.18	8.17	9.01
ActiStar	37.5 ± 1.1	36.7 ± 0.3	36.8 ± 0.9	37.2 ± 0.1	37.1 ± 0.9
	1.53	0.48	1.20	0.13	1.20

[a] all results are presented as starch on an "as is" basis.

[b] on each day, samples of each material were analysed in duplicate.

[c] SD = standard deviation; RSD_r % = repeatability standard deviation.

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4. Conclusion

The method outlined in this document is a robust, accurate and easy method for the measurement of resistant and digestible starch in various matrices. Data presented in this report verifies and validates that this method is fit for the purpose intended, which is summarised below

Validation Summary	
Working range (µg in assay as glucose)	4-100
LOD (ΔA)	0.04
Relative Bias *b*(%) Resistant Starch	- 1.99
Relative Bias *b*(%) Digestible Starch	-1.23
Reproducibility (%CV) Resistant Starch	0.48
Reproducibility (%CV) Digestible Starch	1.31