Megazyme
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 2861220
Fax: + 353 1 2861264

Validation Report: Digestible and Resistant Starch Assy Kit (cat. no. K-DSTRS)

1. Scope

Megazyme's Digestible and Resistant Starch Assy Kit (K-DSTRS) method, is an enzymatic method used for the measurement and analysis of Digestible and Resistant Starch in plant materials, starch samples and other materials. This method is based on the research of Englyst *et al.* with some modifications. This assay kit (K-DSTRS) employs a more physiologically-relevant 4 h incubation step with pancreatic α-amylase/amyloglucosidase than existing methods. This reflects the transit time in the human small intestine.

Rapidly digestible starch (RDS) is that starch which is digested within 20 min. Slowly digestible starch (SDS) is that starch which is digested between 20 and 120 min. A new term, 'Total digestible starch (TDS)' is introduced (and measured) to cover all starch that is digested within 4 h (the average time of residence of food in the human small intestine).

Resistant starch (RS) then, is that starch which is ***not digested*** within 4 h.

2. Planning

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

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 Digestible and Resistant Starch.

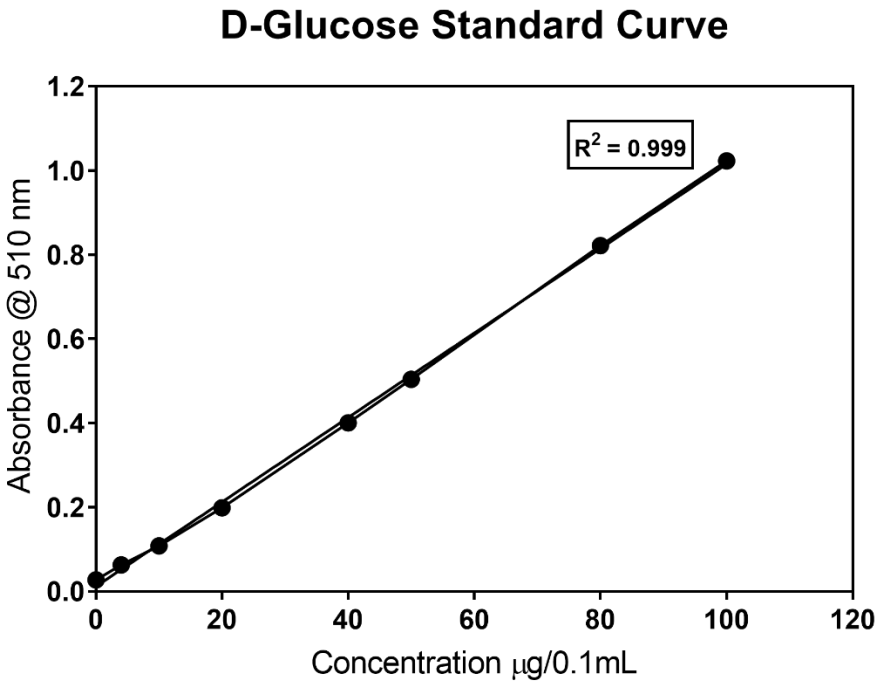
3.2. Working Range

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 D-glucose per assay.

0.1 mL of D-glucose standards at various concentrations were 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.

Megazyme
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 2861220
Fax: + 353 1 2861264

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.



3.3. LOD

If the standard procedure is followed, the smallest differentiating recommended absorbance change (ΔA) is 0.04. This corresponds to ~ 3.1 g/100 g of Digestible starch (RDS / SDS / TDS) and ~ 3.7 g/100 g of Resistant Starch "as is", using a sample weight of 0.5 g and extract volume of 20.5 mL.

The highest ΔA should be lower than the absorbance values obtained for 100 µg of glucose. This is equivalent to ~ 77.5 g/100g of Digestible Starch (RDS / SDS/ TDS) and ~ 92.3 g/100 g 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.

Megazyme
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 2861220
Fax: + 353 1 2861264

3.4 Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with the Digestible and Resistant Starch Assy Kit (K-DSTRS) 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 Digestible and Resistant Starch control which is supplied with the Digestible and Resistant Starch Assy Kit (K-DSTRS), at 8.0 g/100 g of RDS, 16.57 g/100 g SDS, 35.04 g/100 g TDS and 50.02 g/100 g RS content.

Relative Bias *b(%)*

	n	Ref Material (g/100 g)	Mean (g/100 g)	*b*(%)
Rapidly Digestible Starch (RDS)	21	8.0	8.13	1.65
Slowly Digestible Starch (SDS)	21	16.2	16.57	2.28
Total Digestible Starch (TDS)	21	35.9	35.04	-2.39
Resistant Starch (RS)	21	47.4	50.02	5.53

3.5 Precision

This report details the reproducibility of the Digestible and Resistant Starch Assy Kit (K-DSTRS), 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 control is used as the reference material.

Reproducibility

	n	Ref Material (g/100 g)	Mean (g/100 g)	Standard Deviation	%CV
Rapidly Digestible Starch (RDS)	21	8.0	8.13	0.6671	8.20
Slowly Digestible Starch (SDS)	21	16.2	16.57	1.1251	6.79
Total Digestible Starch (TDS)	21	35.9	35.04	1.7879	5.10
Resistant Starch (RS)	21	47.4	50.02	2.2871	4.57

Megazyme
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 2861220
Fax: + 353 1 2861264

Repeatability

The repeatability (%RSD_r) of the Rapid Digestible and Resistant Starch assay method was assessed using 7 milled samples.

For each sample, duplicate extractions were processed and applied to the assay across 4 separate days.

Table 1. The **Rapidly Digested Starch** content of the samples tested covered a working range of 6.9 to 58.8% (w/w). The repeatability (%RSD_r) across this sample data was excellent, less than or equal to 5.52.

Sample	Rapidly Digested Starch, % (w/w)[a], mean[b] ±2 SD, (%RSD_r[c] %)				Interday mean, ±2 SD, (%RSD_r)
	Day 1	Day 2	Day 3	Day 4	
RMS	21 ± 1.4	19.9 ± 0.4	19.2 ± 1.3	19.7 ± 0.2	19.9 ±1.6
	3.29	0.91	3.32	0.46	3.95
Hylon VII	7.4 ± 0.3	6.8 ± 0.5	6.5 ± 0.3	7 ± 0.4	6.9 ±0.8
	2.35	3.61	2.60	2.78	5.52
UB Express Boiled Rice	58.9 ± 2.7	59.3 ± 3.9	57.3 ± 0.7	57.2 ± 2.6	58.2 ±2.9
	2.29	3.32	0.60	2.24	2.48
ActiStar	21.2 ± 0.2	20.6 ± 0.9	20.3 ± 0.8	22.5 ± 0.7	21.2 ±1.9
	0.43	2.11	1.91	1.65	4.38
Garden Peas	13 ± 0.1	12.7 ± 0.3	12.8 ± 0	12.7 ± 0.1	12.8 ±0.4
	0.38	1.23	0.11	0.56	1.37
All Bran	23.6 ± 0.3	24 ± 0.3	24.1 ± 0.2	23.9 ± 0	23.9 ±0.4
	0.56	0.62	0.44	0.03	0.83
Butter Beans	17.9 ± 0.5	18.5 ± 0.2	19.3 ± 1.8	19.1 ± 1.4	18.7 ±1.4
	1.46	0.63	4.75	3.55	3.81

[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.

Megazyme
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 2861220
Fax: + 353 1 2861264

Table 2. The **Slowly Digested Starch** content of the samples tested covered a working range of 1.0 to 49.2% (w/w). The repeatability (%RSD_r) across this sample data set was excellent, less than or equal to 11.63% **for samples containing > 1% SDS** (Table 2) (**Note:** The very high value for All Bran® is a consequence of the very low absolute starch value for this fraction).

Sample	Slowly Digested Starch, % (w/w)[a], mean[b] ±2 SD, (%RSD_r[c] %)				Interday mean, ±2 SD, (%RSD_r)
	Day 1	Day 2	Day 3	Day 4	
RMS	51.1 ± 2.5	48.4 ± 1.7	49.8 ± 1.2	47.3 ± 1.3	49.2 ±3.3
	2.47	1.78	1.24	1.33	3.39
Hylon VII	14.3 ± 0.1	16.5 ± 0.2	16.5 ± 0.5	17.3 ± 0.1	16.2 ±2.4
	0.31	0.75	1.62	0.42	7.42
UB Express Boiled Rice	13.6 ± 3.8	12.3 ± 1.4	11.7 ± 1.3	11.4 ± 0.4	12.2 ±2.4
	13.85	5.61	5.43	1.69	9.77
ActiStar	5.3 ± 1.1	6 ± 0.2	5.7 ± 0	7 ± 0.2	6 ±1.4
	10.44	1.78	0.22	1.62	11.63
Garden Peas	2.6 ± 0.3	2.7 ± 0.6	2.6 ± 0.1	2.5 ± 0.1	2.6 ±0.3
	4.97	11.28	2.44	1.15	5.97
All Bran	1.4 ± 1.1	1.2 ± 0.7	0.7 ± 0.3	0.6 ± 0.3	1 ±0.9
	40.84	30.81	19.00	22.52	45.75
Butter Beans	14.1 ± 2	13.3 ± 0.4	12.7 ± 0.3	12 ± 1.8	13 ±2
	7.21	1.62	1.35	7.44	7.55

[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.

Megazyme
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 2861220
Fax: + 353 1 2861264

Table 3. The **Total Digested Starch** content of the samples tested covered a working range of 16.5 to 80.1% (w/w). The repeatability (%RSD_r) across this sample data set was excellent, less than or equal to 5.47% for all samples.

Sample	Total Digested Starch, % (w/w)[a], mean[b] ±2 SD, (%RSD_r[c] %)				Interday mean, ±2 SD, (%RSD_r)
	Day 1	Day 2	Day 3	Day 4	
RMS	82.1 ± 0.4	79 ± 0.8	79.5 ± 0.4	79.8 ± 1.7	80.1 ±2.7
	0.25	0.54	0.23	1.07	1.67
Hylon VII	33.2 ± 0.9	36.5 ± 1.7	35.7 ± 0.2	38.2 ± 0.6	35.9 ±3.9
	1.34	2.39	0.32	0.84	5.47
UB Express Boiled Rice	72.5 ± 1.3	70.2 ± 0.9	70.8 ± 0.4	71.8 ± 0.1	71.3 ±2
	0.93	0.66	0.29	0.06	1.40
ActiStar	34.6 ± 0.4	34.1 ± 4.1	33.1 ± 0.8	35.6 ± 2.3	34.4 ±2.7
	0.55	6.05	1.21	3.21	3.89
Garden Peas	16.9 ± 0	16.6 ± 0.4	16.3 ± 0.5	16.4 ± 0.1	16.5 ±0.6
	0.04	1.11	1.61	0.17	1.77
All Bran	24.9 ± 1.5	25.2 ± 0.2	25.1 ± 0.1	25.1 ± 0.2	25.1 ±0.6
	2.93	0.32	0.22	0.45	1.27
Butter Beans	34.4 ± 0.6	34.5 ± 0.2	34.7 ± 0	34.9 ± 1.8	34.6 ±0.8
	0.87	0.28	0.05	2.54	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.

Megazyme
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 2861220
Fax: + 353 1 2861264

Table 4. The **Resistant Starch** content of the samples tested covered a working range of 0.3 to 51.8% (w/w). The repeatability (%RSD_r) across this sample data set was excellent, less than or equal to 6.33% for all samples.

Sample	Resistant Starch, % (w/w)[a], mean[b] ±2 SD, (%RSD_r[c] %)				Interday mean, ±2 SD, (%RSD_r)
	Day 1	Day 2	Day 3	Day 4	
RMS	1.9 ± 0.1	2 ± 0.1	1.9 ± 0	2 ± 0.1	2 ±0.1
	1.52	2.77	0.07	2.77	2.47
Hylon VII	48.2 ± 0.1	47.7 ± 1.9	47.1 ± 0.2	46.6 ± 0.6	47.4 ±1.5
	0.14	1.95	0.17	0.60	1.60
UB Express Boiled Rice	2.5 ± 0.4	2.6 ± 0.3	2.6 ± 0.2	2.4 ± 0.2	2.5 ±0.3
	8.06	5.22	4.58	3.28	6.33
ActiStar	52 ± 0.1	52.3 ± 0.3	51 ± 0.1	51.8 ± 0.9	51.8 ±1.1
	0.07	0.25	0.13	0.88	1.05
Garden Peas	7.9 ± 0.4	7.9 ± 0.3	7.6 ± 0.7	8 ± 0.6	7.9 ±0.5
	2.36	1.69	4.55	3.42	3.09
All Bran	0.3 ± 0	0.3 ± 0	0.3 ± 0	0.3 ± 0	0.3 ±0
	4.36	0.95	0.14	3.69	3.96
Butter Beans	3.5 ± 0.1	3.4 ± 0.1	3.5 ± 0.2	3.3 ± 0	3.4 ±0.2
	1.43	1.16	2.24	0.38	3.22

[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.

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:** 4-100 µg in assay as D-glucose
- **LOD (ΔA):** 0.04

	RDS	SDS	TDS	RS
Relative Bias *b*(%)	1.65	2.28	-2.39	5.53
Reproducibility (%CV)	8.2	6.79	5.1	4.57
Repeatability (%RSD_r)	≤ 5.52	≤ 11.63	≤ 5.47	≤ 6.33