Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

Validation Report: Total Starch HK Assay Kit (cat. no. K-TSHK)

1. Scope

Megazyme's Total Starch HK Assay Kit, (K-TSHK) is a modification of AOAC Method 996.11, AACC Method 76-13.01 and RACI Standard Method for the measurement, and analysis of total starch in grains, cereal flours, animal feeds and food products. This Total Starch Assay Kit contains an improved α-amylase that allows the amylase incubations to be performed at pH 5.0 (as well as pH 7.0). The method has been further modified by adjusting the D-glucose determination to a hexokinase/glucose-6-phosphate dehydrogenase/$NADP^+$ UV based format. Total Starch is measured in g/100 g on an "as is" basis or moisture content can be allowed for, giving total starch content in g/100 g on a "dry weight basis".

2. Planning

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

3. Performance characteristics

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

3.1. Selectivity

The assay is specific for α-glucans (including starch, glycogen, phytoglycogen and non-resistant maltodextrins).
Interfering substances in the sample being analysed can be identified by including an internal standard. Quantitative recovery of this standard would be expected. Losses in sample handling and extraction are identified by performing recovery experiments, i.e. by adding D-glucose to the sample in the initial extraction steps.

3.2. Working Range

The working range of the Total Starch HK Assay Kit (K-TSHK) is determined by the D-glucose control provided in the kit. The glucose measurement is linear between 4 to 80 µg of D-glucose per assay.

0.1 mL of D-Glucose standard was used as sample, with a range of concentrations (0.04-0.8 g/L D-Glucose) which corresponds to 4-80 µg of D-Glucose per cuvette. Absorbance A_2 was read after 5 min, at 340 nm and at 25°C as recommended in the procedure.

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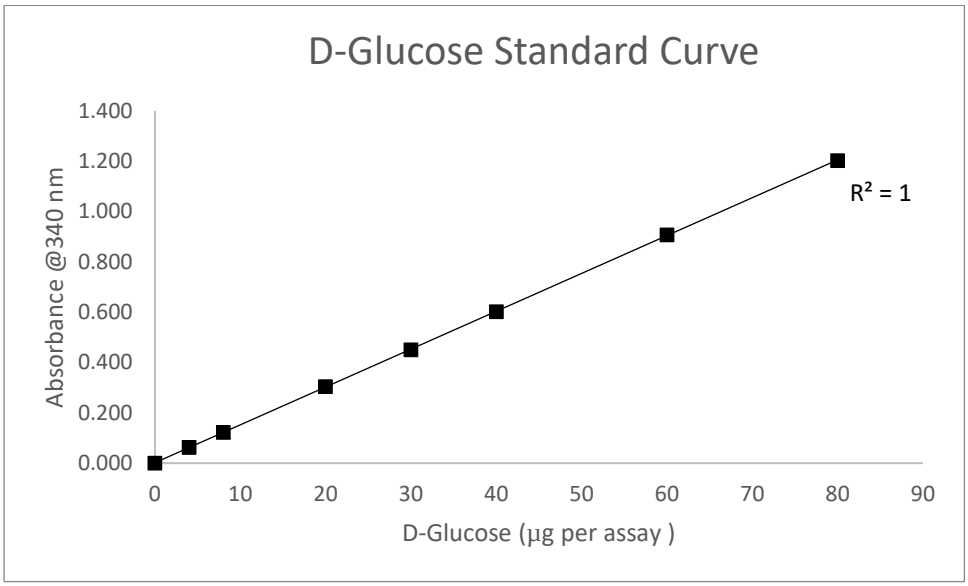
D-Glucose Concentration [µg/assay]	ΔA_{340nm}
0	0.000
4	0.063
8	0.122
20	0.305
30	0.451
40	0.602
60	0.908
80	1.203



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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 0.649 mg/L (or 0.584 mg starch/L), which is derived from an absorbance difference of 0.020 with the maximum sample volume of 2.00 mL

The **calculated limit of detection (LOD)** and the **calculated limit of quantification (LOQ)** for this report purpose is based on the analysis of samples that have been taken through the standard procedure of the Total Starch HK Assay Kit (K-TSHK).

- The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as 3 x σ of the blank sample solution absorbance and 10 x σ of the blank sample solution absorbance, respectively, where σ is the standard deviation of the absorbance values from 10 replicates.

- For Total Starch HK Assay Kit (K-TSHK)

  LOD – For 2.0 mL of sample (maximum volume)
  D-Glucose = 0.06 mg/L (which is equivalent to 0.05 mg/L starch)

  LOQ – For 2.0 mL of sample (maximum volume)
  D-Glucose = 0.19 mg/L (which is equivalent to 0.169 mg/L starch)

* **Note**: The above detection limits are for samples as used in the assay, after any sample preparation, if required. The dilution used in pre-treatment must be accounted for while establishing the detection limits for specific samples.

3.4. Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with Total Starch HK Assay Kit (K-TSHK) 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 a regular maize starch control supplied with the Total Starch HK Assay Kit (K-TSHK) with a starch content of 93% w/w on a dry weight basis.

Relative Bias *b*(%)

	n	Ref Material (% w/w)	Mean (U/mL)	*b*(%)
Total Starch	31	93	93.52	0.55

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3.5. Precision

This report details the reproducibility of the Total Starch HK Assay Kit (K-TSHK), 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 (%w/w)	Mean (%w/w)	Standard Deviation	%CV
Total Starch	31	93	93.52	3.01	3.22

4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of total starch in various matrices and has been used for many years. Data presented in this report verifies and validates that this method is fit for the purpose intended, which is summarised below.

Validation Summary	D-Glucose
Working range (µg in cuvette)	4-80
LOD (mg/L)	0.10
LOQ (mg/L)	0.39
Relative Bias *b* (% using maize control)	0.55
Reproducibility (%CV using maize control)	3.22