MALT α-AMYLASE / β-AMYLASE STANDARD

08/23

Non-recombinant

E-MAST

α-Amylase

EC: 3.2.1.1
Synonyms: alpha-amylase
CAZy Family: GH13
CAS: 9000-90-2

β-Amylase

EC: 3.2.1.2
Synonyms: beta-amylase
CAZy Family: GH14
CAS: 9000-91-3

Refer to the product lot number Certificate of Analysis for lot specific properties.

E-MAST is a malt extract in which the "Apparent" Diastatic Power (°Lintner) and levels of α-amylase and β-amylase have been standardised. The preparation is designed for use as a standard in the determination of α-amylase and diastatic power USING SKALAR Continuous Flow Analyser Equipment and β-Limit Dextrin (**P-BLDX**).

PROPERTIES

1. **STANDARDISATION:**

   For use in Skalar and Gallery analysers, **E-MAST** Stock Standard solution (apparent DP °ASBC 14,720) is diluted 50-fold [2 mL of E-MAST to 98 mL of 0.5% NaCl containing 5 mM calcium chloride (0.75 g $CaCl_2.2H_2O$/L)] to give a Working Stock solution with an apparent DP of 295 °ASBC. Dilute this solution further with the NaCl/$CaCl_2$ solution to concentrations of 20-100% (See Figure 1). These solutions are used to standardise the SKALAR and Gallery automated machines.

   To determine the activity of **E-MAST** using ASBC Malt-6, dilute the Working Stock solution (295 °ASBC) 10-fold (5 mL of Working Stock solution adjusted to 50 mL in a volumetric flask with 0.5%NaCl containing 5 mM calcium chloride) to obtain an activity of 29.5 °ASBC. Dilute this solution further by mixing 5, 4, 3, 2 or 1 mL of the diluted solution (29.5 °ASBC) with 0, 1, 2, 3 or 4 mL of 0.5% NaCl containing 5 mM calcium chloride. Determine diastase activity using the standard ASBC Malt-6 procedure scaled down 10-fold, as follows: incubate 1 mL of the diluted E-MAST solutions with 20 mL of 2% w/v soluble starch (pH 4.6) at 20°C for 30 min. Terminate the reaction by adding 2 mL of 0.5 N NaOH and adjust the volume to 25 mL. Determine the level of reducing sugar produced using the ferricyanide procedure as per ASBC Malt-6, and calculate activity as described. The factor "23" as employed in the activity determination, includes a 5-fold dilution, which must be accounted for when determining DP.

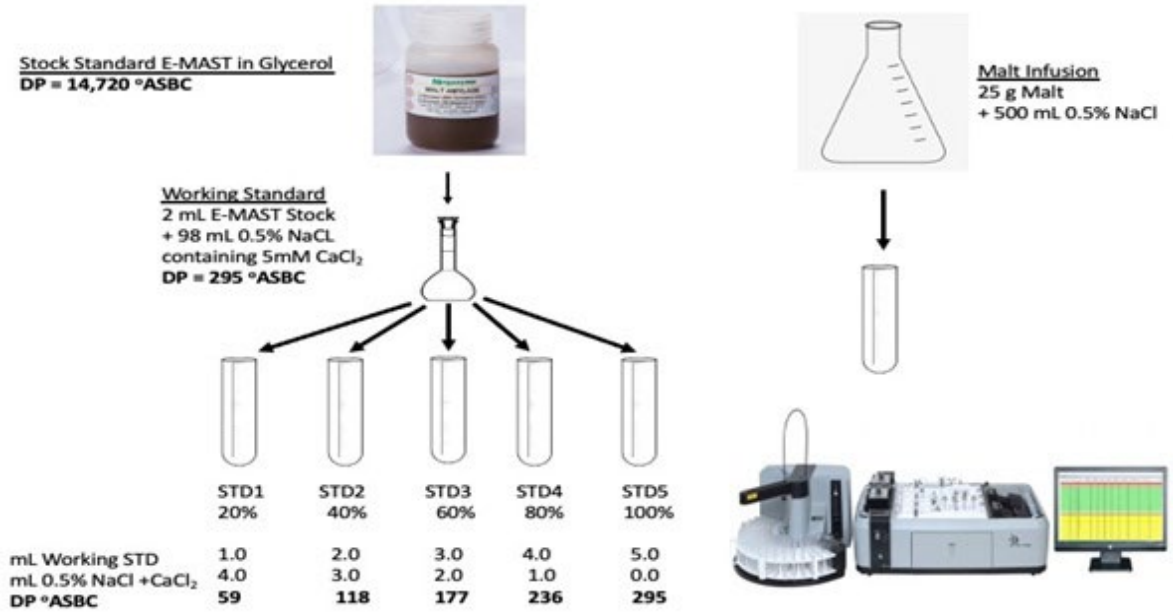

Figure 1.

For example:

DP °ASBC = (B-A) x 23 x dilution = (8.34 – 1.94) x 23 x 50 x 1/5 x 10 = 14,720

NOTE: the initial dilution of 50-fold includes a 5-fold dilution which is already included in the factor "23", meaning that the dilution is actually 10-fold. The diastase is further diluted 10-fold to bring on scale for assay.

2. STORAGE CONDITIONS:

The enzyme is supplied as a stabilised liquid in 50% (v/v) glycerol and should be stored at 4°C or below -10°C for longer periods.

Standardisation of Malt Amylase (Megazyme E-MAST & E-MASTP) for Diastatic Power as described by ASBC, IoB and EBC

Background: Megazyme supplies a malt amylase solution (**E-MAST**) and malt amylase powder (**E-MASTP**) with defined α-amylase and β-amylase units for use as a control in the various ASBC and EBC methods for the measurement of Diastatic Power (DP). The two enzyme activities in E-MAST and E-MASTP are measured using the Ceralpha (**K-CERA**) and Betamyl-3 (**K-BETA3**) methods available from Neogen and are expressed as Ceralpha Units for α-amylase and Betamyl-3 Units for β-amylase respectively. However, there is a preference for this material to be supplied in degrees Lintner (°Lintner) and in Windisch-Kolbach units (°WK). The original method used by ASBC to measure DP was based on Fehling's reagent and expressed DP values as °Lintner. In 1937[1] it was proposed that a new standardised method for DP based on ferricyanide should be used. The ferricyanide method expresses DP as mL of ferricyanide equivalents. Obviously, the DP values as expressed in the Fehling's reagent method as °Lintner did not match with values obtained with the ferricyanide method, which are as "mL of ferricyanide" used in the titration, so a conversion factor was

needed. In the initial study by Anderson and Sallans (1937), a factor of 18 was proposed. Subsequently, an interlaboratory evaluation was undertaken by both ASBC and AACC in which some laboratories measured DP using the old Fehling's reagent method while others used the novel ferricyanide method. The absolute DP values obtained using the two methods on the same malt samples, on average differed by a factor of 23.[2] The difference between the factor of 18 obtained by Anderson and Sallans[1] and that obtained from the study ASBC/AACC study (23) is due to the different volumes of final diastasis solution used in the two assays;
200 mL in the Anderson and Sallans[1] procedure and 250 mL in the ASBC/AACC procedure,[3] so the factor is calculated as 18 x 250/200 = 22.5. Both ASBC and AACC recommended the adoption of this empirical conversion factor.

In the ASBC method for measuring DP,[3] a ***defined*** procedure for extraction and dilution of malt samples is described and the "correction factor" of 23 is used to relate titration values obtained with the ferricyanide method back to the original oLintner obtained with Fehling's reagent.[1,4,5]

Degrees Lintner (°Lintner) is a unit used to measure the ability of a malt to hydrolyse starch to reducing sugars, that is, its dextrinising power (DP). The original definition of °Lintner was first described in 1886[6] and subsequently reported by JECFA, the Joint FAO/WHO Expert Committee on Food Additives as follows:
"A malt has a diastatic power of 100°Lintner if 0.1 mL of a clear 5% infusion of the malt, acting on 100 mL of a 2% starch solution at 20°C (and ~ pH 4.6) for one hour, produces sufficient reducing sugars to reduce completely 5 mL of Fehlings solution."
(Note: the original Lintner method refers to 10 mL of 2% starch solution, so the value of 100 mL in the JECFA report is incorrect – the value should be 10 mL).

In the current ASBC method for measurement of diastatic power (Malt-6),[3] 25 g of malt is extracted with 500 mL of 0.5% sodium chloride solution at 20°C for 2.5 h. This solution is filtered and 20 mL is diluted to 100 mL with 0.5% sodium chloride solution and mixed thoroughly (i.e. equivalent to 1 g malt per 20 mL). The diastatic power of 10 mL of this solution is determined by transferring the 10 mL to a flask containing 200 mL of starch (2% w/v) in ~ 20 mM sodium acetate buffer (pH 4.6) and incubating at 20°C for 30 min. The reaction is terminated by adding 20 mL of 0.5 N sodium hydroxide solution and the volume is adjusted to 250 mL. An aliquot of this solution (5 mL) is removed for the analysis of the extent of reaction using the ferricyanide method. A reaction blank is prepared by adding 20 mL of 0.5 N sodium hydroxide solution to 10 mL of the diluted malt extract before adding 200 mL of the starch solution. This is mixed well and the solution made up with 250 mL of distilled water. An aliquot (5 mL) is removed for analysis by the ferricyanide method. Diastatic power of the malt is calculated in degrees Lintner (°Lintner) according to the following formula:

Diastatic power, °ASBC (as is) = (B – A) x 23.

$$\text{Diastatic power, °ASBC (dry basis)} = \frac{\text{°ASBC (as is)} \times 100}{100 - M}$$

where:
B = mL of sodium thiosulphate used for blank correction titration
A = mL of sodium thiosulphate used for direct titration of the incubation mixture.
23* = conversion factor specific for the procedure outlined.4
M = % moisture in the malt.

* Collaborative work performed by members of ASBC[2] established that, when the conditions of the above described method are maintained, the volume of 0.05 N ferricyanide solution corrected for the blank and multiplied by 23 gives the diastatic power in °Lintner "as is".

Following the EBC method for DP (method 4.12.1),[7] diastatic activity is expressed in Windisch-Kolbach units (°WK). ASBC and EBC DP values are interconverted using the following equations:

$$^{o}\text{Lintner} = \frac{^{o}\text{WK} + 16}{3.5}$$

$$^{o}\text{WK} = (^{o}\text{Lintner} \times 3.5) - 16.$$

3. REFERENCES:

1. Anderson, J. A. & Sallans, H. R. (1937). Determination of the diastatic power of malt in degrees Lintner by means of a ferricyanide reagent. Can. J. Res., 15c(2), 70-77.
2. ASBC Report of Subcommittee on diastatic power determination, Proc. 1941, p. 90; Proc. 1942, p. 124.
3. ASBC Methods of Analysis, Diastatic Power Method Malt-6, 14th Edition.
4. Dickson, A. D. (1944). J. AOAC Int., 27 (3), 374-375.
5. Dickson, A. D. (1942). Report of the malt analysis standardisation committee. Cereal Chem., 19, 249-251.
6. Lintner, C. J. (1886). Studien über Diastase. J. Prakt. Chem., 34, 378-394.
7. EBC Official Methods of Analysis (2018) Method 4.12.1 Diastatic power of malt by spectrophotometry (Manual Method).
8. IoB Methods. J. Inst. Brew (1971) 6. Determination of diastatic activity. B. using ferricyanide titration, 77, 192-195.