MALT α-AMYLASE / β-AMYLASE STANDARD (LOT 170812)

E-MASTP

E-MASTP is a malt extract in which the 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 Megazyme β-Limit Dextrin (**P-BLDX**).

1. KEY ACTIVITIES:

Enzyme	Activity (U/g "as is")
α-Amylase (Ceralpha reagent)	14,300
β-Amylase (Betamyl-3 reagent)	1,800
β-Amylase (starch substrate)	72,180
Diastatic Power (°ASBC)	9,000
Diastatic Power (°IoB)	9,500
Diastatic Power (°WK EBC)	32,320
Maltose Value (30°C) (MV30)	18,640

2. PREPARATION FOR USE:

Before use, store the bottle of E-MASTP in a bottle with desiccator and allow to warm to room temperature.

Accurately weigh 1 g of powder into 100 mL of 0.5% w/v NaCl and dissolve with stirring. Store cool and use on the day of preparation.

3. STORAGE CONDITIONS:

The enzyme is supplied as a freeze dried powder and should be stored in the presence of a desicant and below -10°C for longer periods.

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

27th November 2020

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 Megazyme 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 instead. This new ferricyanide method expressed 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. Following the initial 1937 study, 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. Therefore, both ASBC and AACC recommended the adoption of this empirical conversion factor.

In the ASBC method for measuring DP[2], 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 °Lintner obtained with Fehling's reagent.[1,3,4]

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[5] 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)[2], 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 250mL 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, °Lintner (as is) = (B – A) x 23.

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

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 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)[6], diastatic activity is expressed in Windisch-Kolbach units (^{o}WK). ASBC and EBC DP values are interconverted using the following equations:

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

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

Megazyme Malt Amylase Control (E-MASTP; Lot 170812).

α-amylase (Ceralpha) = 14,300 U/g

β-amylase (Betamyl-3) = 1,800 U/g

To determine ^{o}L ASBC for E-MASTP, 1 g of powder was dissolved in 20 mL of 0.5% NaCl solution {the same ratio used in preparing malt extracts [25 g malt/500 mL salt solution (1:20)] and then further diluted 5-fold (20 mL of extract to 100 mL of salt solution); total of 100 mL of solution per g of malt}. For E-MASTP, this solution is then diluted a further 5 fold to be consistent with the ASBC extraction/dilution protocol, but then further diluted a further 100-fold to get the activity on scale, and then assayed according to the standard ASBC method for DP (Malt-6) (i.e. 10 mL of malt amylase solution added to 200 mL of 2% starch solution etc). Diastatic power for malt (as is) is calculated as (B-A) x 23. This calculation is based on strict extraction and assay conditions. In this assay, malt is extracted with sodium chloride solution in a ratio of 20-fold (NaCl solution relative to malt) and this solution is further diluted 5-fold; giving a total ratio of extraction solution to malt of 100. B is the mL of sodium thiosulphate used for blank correction titration, and A is the mL of sodium thiosulphate used for direct titration of the incubation mixture. 23 is a conversion factor between ferricyanide equivalents and traditional ^{o}Lintner determined with Fehling's solution (discussed separately), this conversion factor is specific for the procedure outlined. To standardise malt amylase control solution (**E-MAST**) and control powder (**E-MASTP**), the extract needs to be diluted further to get the assay on scale. The determined ^{o}Lintner values are thus multiplied by these further dilution factors.

The IoB DP reported here was obtained using the Official IoB method[8], and EBC values determined using the Iodiometric method (Method 4.12.1)[6].

E-MASTP (Lot 170812).

ASBC Diastatic Power = 9,000 ºL ASBC.

IoB Diastatic Power = 9,500 ºL IoB.

EBC Diastatic Power = 32,320 ºWK.

Maltose Value (30ºC) = 18,640.

(α-Amylase; Ceralpha = 14,300 U/g) (β-Amylase; Betamyl-3 = 1,800 U/g).

(Maltose Value = mg of maltose released per g of malt/min at 30˚C. Details of this assay procedure are provided separately).

1 g of this control is dissolved in 100 mL of saline solution and then further diluted 100-fold to bring the preparation on scale for assay. At a dilution of 100-fold, the determined diastatic power is 90ºLintner.

Diastatic power (DP) of the original E-MASTP powder is then

100 x 90 ºL ASBC= 9,000 ºL ASBC.

100 x 95 ºL IoB= 9,500 ºL IoB.

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 Methods of Analysis, Diastatic Power Method Malt-6, 14th Edition.
3. Dickson, A. D. (1944). *J. AOAC Int.*, 27 (3), 374-375.
4. Dickson, A. D. (1942). Report of the malt analysis standardisation committee. *Cereal Chem.*, 19, 249-251.
5. Lintner, C. J. (1886). Studien über Diastase. *J. Prakt. Chem*., 34, 378-394.
6. EBC Official Methods of Analysis (2018) Method 4.12.1 Diastatic power of malt by spectrophotometry (Manual Method).
7. IoB Methods. *J. Inst. Brew* (1971) 6. Determination of diastatic activity. B. using ferricyanide titration, **77**, 192-195.

BMcC Dec 2020