

# MALT CARBOHYDRASE

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## SYNONYMS

Malt

## SOURCES

Malt is the product of controlled germination of barley

### Active principles

1. alpha-Amylase (glycogenase, diastase)
2.  $\beta$ -Amylase (glycogenase, diastase)

### Systematic names and numbers

1. 1,4-alpha-D-Glucan glucanohydrolase (EC 3.2.1.1)
2. 1,4-alpha-D-Glucan maltohydrolase (EC 3.2.1.2)

### Reactions catalyzed

1. Hydrolysis of 1,4-alpha-glucosidic linkages in polysaccharides, (starch, glycogen) yielding dextrans and oligo- and monosaccharides.
2. Hydrolysis of 1,4-alpha-glucosidic linkages in polysaccharides, (starch, glycogen) yielding successively maltose units from the non-reducing ends of the chains.

## FUNCTIONAL USES

Enzyme preparation  
Used in brewing, baking, manufacture of alcoholic beverages and manufacture of syrups.

## GENERAL SPECIFICATIONS

Must conform to the *General Specifications for Enzyme Preparations used in Food Processing* (see Volume Introduction)

## CHARACTERISTICS

### IDENTIFICATION

#### alpha-Amylase activity (Vol. 4)

The sample shows alpha-amylase activity

#### alpha- and $\beta$ -amylase activity

The sample shows Diastatic power  
See description under TESTS

## TESTS

### IDENTIFICATION TESTS

#### Diastatic power

Determination of a diastatic power of malt (combined activity of alpha- and  $\beta$ -amylase) according to Amer. Soc. Brewing Chem., 6th ed., 162 (1958).

#### Reagents

##### For Digestion of Starch Solution

- Acetate buffer solution: Dissolve 68 g of sodium acetate ( $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ ), reagent grade, in 500 ml of one N acetic acid and make the solution up to one litre with distilled water.
- Sodium hydroxide solution: 0.5 N
- Special starch: Starch manufactured specifically for diastatic power

determination is available from Merck & Co., Rahway, New Jersey. It is designated, "Soluble Starch Merck, according to Lintner, Special for diastatic power determination". No other manufacturer of this special starch is known to the society.

When purchasing new batches of starch, test them in parallel with the lot in use. Variations of more than  $\pm 3^\circ$  Diastatic Power in the averages of a series of parallel tests indicate an unsuitable batch of starch.

#### For Reducing Substances, Ferricyanide Procedure

- Acetic acid-salt solution: Dissolve 70 g of potassium chloride and 20 g of crystallized zinc sulfate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) in distilled water, add 200 ml of glacial acetic acid and make up to one liter with distilled water.

- Alkaline ferricyanide solution: 0.05N. Dissolve 16.5 g of potassium ferricyanide ( $\text{K}_3\text{Fe}(\text{CN})_6$ ) and 22 g of anhydrous sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) in distilled water and make up to one liter. Keep the solution in the dark away from the light.

- Standardization of the Alkaline Ferricyanide Solution: With an accurate pipette add 10 ml ( $\pm 0.05$  ml) of the alkaline ferricyanide solution to 25 ml of acetic acid salt solution. Add one ml of potassium iodide solution, and two ml of starch solution (see Preparation of Starch Solution, below). Titrate with 0.05N sodium thiosulfate solution, using a 10 ml of semi-micro burette, until the blue starch iodide colour is discharged. Calculate the normality of the alkaline ferricyanide solution, which should lie between 0.0495 and 0.0505.

- Potassium iodide solution: Dissolve 50 g of potassium iodide in 50 ml of distilled water and make up to 100 ml. Add one or two drops of concentrated sodium hydroxide solution. The final solution should be colourless.

- Sodium thiosulfate solution: 0.05N. Dissolve 12.41 g of dry, large clear crystals of sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) and 3.8 g of borax ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) (used as a preservative) in 100 to 200 ml of distilled water and make up to one liter. Standardize the sodium thiosulfate solution with potassium dichromate.

Dry pure  $\text{K}_2\text{Cr}_2\text{O}_7$  (small crystals) for 1 h or more at  $105^\circ$  and weigh 0.1000 g ( $\pm 0.0005$  g) portions for the standardization into 250 ml Erlenmeyer flasks. Dissolve the crystals in 50 ml of water, add two ml of potassium iodide solution and eight ml of concentrated hydrochloric acid solution. Mix thoroughly and titrate with the thiosulfate solution, swirling the mixtures constantly until the brown colour changes to a yellowish green. Add one or two ml of starch solution and continue the titration until the colour changes from blue to light green. About 40 ml of 0.05N thiosulfate solution will be used for the titration. Calculate the normality of the thiosulfate solution by use of the formula:

$$N = \frac{1,000 w}{49.035 v}$$

where

N = the normality of the thiosulfate solution

v = the volume of the thiosulfate solution used for the titration,

w = the weight of  $\text{K}_2\text{Cr}_2\text{O}_7$  used

49.035 = the iodimetric equivalent weight of  $\text{K}_2\text{Cr}_2\text{O}_7$

The normality of the sodium thiosulfate solution should lie between 0.0495 and 0.0505.

#### For Reducing Substances, Fehling's Solution Procedure

- Methylene blue, one per cent aqueous solution
  - Soxhlet's modification of Fehling's Solution. Prepare by mixing immediately before use equal volumes of (A) and (B):
    - (A) Copper sulfate solution: Dissolve 34.639 g of copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) in water, dilute to 500 ml and filter through prepared asbestos.
    - (B) Alkaline tartrate solution: Dissolve 173 g of Rochelle salt (sodium potassium tartrate) and 50 g of sodium hydroxide in water, dilute to 500 ml, allow to stand for two days and filter through prepared asbestos.
- Standardize the mixed Fehling's solution by using an invert sugar solution, according to the LANE-EYNON Volumetric method, or by using pure dextrose according to the following method:
- Instead of preparing a solution of invert sugar, pure dextrose may be used to standardize the Soxhlet Solution. Dry pure, reagent grade, anhydrous dextrose ( $\text{C}_2\text{H}_{12}\text{O}_6$ ) for 1.5 h at  $103^\circ$ , weigh out 4.82 g, dissolve it in distilled water and make the volume up to one litre. 25 ml of the solution contain exactly 120.5 mg of dextrose and should exactly reduce 25 ml of Soxhlet Solution (Table 5, A.S.B.C. "Tables Related to Determinations on Wort, Beer, and Brewing Sugars and Syrups").
- If the standardization is based on 10 ml of Soxhlet Solution and 10 ml of distilled water, prepare the dextrose solution to contain 1.265 g of dextrose in 40 ml which should exactly reduce 10 ml of Soxhlet Solution (Table of "Factors for 10 ml Soxhlet solution to be used in connection with Lane-Eynon general volumetric method", Official Methods of Analysis, A.O.A.C.).
- Sodium chloride. 0.5 per cent. Dissolve 5.0 g of sodium chloride reagent grade in freshly distilled water and dilute the solution to one liter.

#### Apparatus

(MALT-4 makes reference to Amer. Soc. Brewing Chem., 6th ed., Method MALT-4)

- Balance: See MALT-4, EXTRACT, Apparatus (b)
- Electric hot plate
- Filter paper: See MALT-4, EXTRACT, Apparatus (d)
- Funnels: 20 cm. See MALT-4, EXTRACT, Apparatus (f)
- Glassware: Burettes, 20 ml semimicro-micro; 50 ml Flasks, Erlenmeyer, 125 ml; 250 ml; Flasks, volumetric, 100 ml; 250 ml; one liter; Pipettes, 10 ml (precision); 20 ml; 200 ml (fast)
- Infusion flasks, 1 L Erlenmeyer flask or glass-stoppered bottle is suitable.
- Mash beaker: See MALT-4, EXTRACT, Apparatus (i)
- Mill, fine Grind: See MALT-4, EXTRACT, Apparatus (k)
- Stopwatch
- Water bath,  $20 \pm 0.2^\circ$
- Water bath, boiling water.

Wash all glassware to be subsequently used in the determination first with chromic acid cleaning solution, then rinse with ordinary tap water not less than four times, and finally rinse with distilled water at least twice.

Thoroughly drain the infusion flasks.

#### Digestion of the Starch Solution

Preparation of the Special Starch Solution: Prepare a 2% solution of

special starch by weighing the equivalent of 2 g of starch dry basis, for each 100 ml of solution required, and macerating it with not over 5% of the final volume of cold freshly distilled water, stirring it into a smooth thin paste. Add the starch paste slowly with constant stirring to vigorously boiling freshly distilled water at such a rate that boiling does not cease. The volume of boiling water should be not less than 75% of the final volume of starch solution. Continue boiling the solution for 2 min after the last of the paste is added, then quickly add 10% of the final volume of cold freshly distilled water to the boiling solution and transfer it quantitatively to a glass-stoppered volumetric flask. Mix by inverting the flask, wash down the neck of the flask with a little distilled water and cool the solution to 20°.

When cooled add 2 ml of acetate buffer solution for each 100 ml of final volume and make to the mark with distilled water. Mix again by inverting the flask. Keep the flask tightly stoppered and at 20° until used.

#### Preparation of the Malt Infusion

Grind separately not over 25.5 g of malt according to the method for fine grinding of malt for determination of extract (see Amer. Soc. Brewing Chem., 6th ed., Method MALT-4, EXTRACT, Preparation of the Sample for Mashing, Fine Grinding). Collect the finely ground malt in a mash beaker. Carefully brush malt particles remaining in the mill into the mash beaker. Without delay, place the mash beaker with its contents on the balance, adjust the weight of ground malt to  $25 \pm 0.05$  g and transfer it to the infusion flask. Add 500 ml of 0.5% sodium chloride solution, close the flask, swirl, and note the time. Let the infusion stand for 2.5 h at  $20 \pm 0.2^\circ$  agitating it by rotation at 20 min intervals. The infusion flask must not be mixed by inverting it, and the quantity of grist left adhering to the inner walls of the flask as a result of agitation must be as small as possible. Gentle swirling of the contents of the flask without splashing it against the walls will give sufficient mixing. At the end of 2.5 h, filter the infusion through a 32 cm fluted filter in a 20 cm funnel. Return the first 50 ml of filtrate to the filter. Collect the filtrate until 3 h have elapsed after the time the sodium chloride solution and the ground malt were first mixed. Place a watch glass over the funnel and a suitable cover around the stem and over the receiver to reduce evaporation losses during the filtration.

#### Diastasis

When the filtration of the infusion is completed, transfer immediately 20 ml of the filtrate to a 100 ml volumetric flask and dilute it to the mark with 0.5% sodium chloride solution. Mix well, and with a pipette, transfer 10 ml of the diluted extract to a 250 ml volumetric flask and bring it to 20°. Add 200 ml of buffered starch solution at 20° to the flask from a fast flowing pipette and start a stopwatch the instant the addition begins. Mix by rotating the flask during the addition. Maintain the "starch infusion" mixture at  $20 \pm 0.1^\circ$  for exactly 30 min from the time the addition of the starch solution was begun. At the end of 30 min add 20 ml of 0.5N sodium hydroxide rapidly and mix by inverting the flask. Make the volume up to 250 ml at 20° with distilled water and mix well.

#### Preparation of the Blank Correction Solution

Prepare a blank solution by adding 20 ml of 0.5 N sodium hydroxide to the 10 ml of diluted malt extract before adding the 200 ml of starch solution.

Otherwise treat the blank solution in exactly the same way as the starch solution actually undergoing diastasis.

### Determination of Reducing Substances, Ferricyanide Procedure

#### Titration (A)

With a pipette add  $5 \pm 0.05$  ml of the digested starch solution to a 125 ml-Erlenmeyer flask. Add  $10 \pm 0.05$  ml of alkaline ferricyanide reagent, mix well, and immerse the flask in a vigorously boiling water bath for exactly 20 min by a stopwatch. The level of the boiling water should be slightly above the level of the mixture in the flask. After 20 min in the bath remove the flask and cool under running water to room temperature. Add 25 ml of acetic acid-salt solution and one ml of potassium iodide solution. Mix well and titrate with the 0.05 N sodium thiosulfate solution to the complete disappearance of the blue colour. Use a 10 ml-semimicroburette for the titration and read it to 0.01 ml. Call the volume of sodium thiosulfate solution used for the titration, "A".

#### The Blank Correction (B)

Add with a pipette 5 ml of the blank correction solution to a 125 ml-Erlenmeyer flask, add  $10 \pm 0.05$  ml of 0.05N alkaline ferricyanide solution. Immerse the flask in boiling water for 20 min, cool, and titrate it in exactly the same way as for the direct titration above. Call the number of ml of 0.05 N sodium thiosulfate solution required for the blank titration, "B".

#### Calculation

Calculate the diastatic power of the malt in degrees by use of the formulas:

$$\text{Diastatic Power}^{\circ} \text{ (as is)} = (B - A) \times 23$$

$$\text{Diastatic Power}^{\circ} \text{ (dry basis)} = \frac{(\text{as is}) \times 100}{100 - M}$$

where

B = ml of sodium thiosulfate used for the blank correction titration

A = ml of sodium thiosulfate used for the direct titration

M = per cent moisture in the malt

Report Diastatic Power as Degrees to the nearest whole number.

Example

M = 4.1%

B = 9.65 ml of 0.05 N  $\text{Na}_2\text{S}_2\text{O}_3$  solution

A = 5.04 ml of 0.05 N  $\text{Na}_2\text{S}_2\text{O}_3$  solution

$$\text{Diastatic Power}^{\circ} \text{ (as is)} = (9.65 - 5.04) \times 23 = 106$$

$$\text{Diastatic Power}^{\circ} \text{ (dry basis)} = \frac{106 \times 100}{100 - 4.1} = 111$$

### Fehling's (or Soxhlet) Solution Procedure

#### Titration (A)

Mix 10.0 ml of Soxhlet solution and 10 ml of water in a 200 ml-Erlenmeyer flask and bring it to a boil, preferably on an electric hot plate, or over a small gas flame. Add, from a burette, about two-thirds of the volume of digested starch solution probably necessary for complete reduction of the

copper, and boil for 15 to 20 sec, rotating the flask constantly. Remove from the heat, and, if the liquid is decidedly blue, add more digested starch solution, boil for about 10 sec, and again observe the colour. When the blue colour is almost discharged, and after boiling gently for about 2 min, add three drops of a 1% methylene blue solution. Continue boiling, and adding more diluted starch solution until 0.1 ml or even one drop completely discharges the blue colour. Near the end-point the colour is violet lavender.

After the methylene blue indicator is added do not interrupt the boiling. The flask must remain filled with steam to prevent entrance of air. Titrate (a burette with an S- or Z-shaped extension to the tip that will extend over the boiling flask is an advantage in this titration) into the gently simmering solution in flask. On cooling the blue colour usually returns. Repeat the titration, adding at once almost the whole amount of the digested starch solution required and finish off the titration drop by drop as given above. Call the volume of digested starch solution required to reach the end-point in the second titration "A".

#### The Blank Correction (B)

Add to 10 ml of Soxhlet solution and 10 ml of water a volume of blank correction solution equal to A, the final value of digested starch solution required in the direct titration. Boil the mixture and again determine the end point by titrating with the digested starch solution as in The Titration (A), above. Call the volume of digested starch solution used to reach the end-point "B"

#### Calculation

Calculate the diastatic power of malt in degrees by use of the formulas:

$$\text{Diastatic Power}^{\circ} \text{ (as is)} = \frac{5,000}{A} \times \frac{B}{A}$$

$$\text{Diastatic Power}^{\circ} \text{ (dry basis)} = \frac{\text{(as is)} \times 100}{100 - M}$$

where

A = ml of digested starch solution used in the direct titration

B = ml of digested starch solution used in the blank correction titration

M = per cent moisture in the malt

In the formula for Diastatic Power<sup>o</sup> (as is), 5,000/A is the apparent diastatic power which must be corrected for the effect of the reducing substances in the starch solution and in the salt infusion prior to diastasis.

The fraction B/A is the correction factor for these effects.

Report Diastatic Power as Degrees to the nearest whole number.

Example

A = 44.2 ml of digested starch solution in the direct titration

B = 41.3 ml of digested starch solution in the blank correction titration

$$\text{Diastatic Power}^{\circ} \text{ (as is)} = \frac{5000}{A} \times \frac{41.3}{44.2} = 106$$

$$\text{Diastatic Power}^{\circ} \text{ (dry basis)} = \frac{106 \times 100}{100 - 4.1} = 111$$

### Notes

Collaborative work was established that when the conditions of the Ferricyanide Procedure are maintained, the volume of 0.05 Normal ferricyanide solution corrected for the blank and multiplied by 23 gives the diastatic power in Degrees "as is".

A definition of "Degrees Diastatic Power" is implied in the foregoing instructions for determination of diastatic power. The original definition of "Degrees Lintner" is as follows: A malt has a diastatic power of 100°L, if 0.1 cc of a clear 5% infusion of the malt, acting on 100 cc of a 2% starch solution at 20° for one h, produces sufficient reducing sugars to reduce completely 5 cc of Fehling's solution. Since the original Lintner method used a malt infusion prepared with distilled water, while the method given above uses a 0.5% sodium chloride solution for extraction of the diastase, the "Lintner" of the diastatic power designation was dropped. The use of 0.5% sodium chloride solution and reporting of diastatic power in the form, "Diastatic Power-120°" became effective January 1, 1957.

### References

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