PREPARATION:
Beta-limit dextrin is produced by treatment of lintnerised maize starch with pure $\beta$-amylase to the limit of hydrolysis. The $\beta$-amylase is then inactivated and free maltose is removed by ultrafiltration. The content of maltose in the original hydrolysate is about 50%. This is reduced to about 1% by ultrafiltration. The product is then dried.

PROPERTIES:
Beta-limit dextrin as supplied by Megazyme is not hygroscopic as is the case for other commercially available materials. The reason for this is that with the other products, the maltose has not been removed.

USE:
When using the Megazyme $\beta$-limit dextrin in Farrand, AACC (SKB Units) or ASBC (Dextrinising Units, DU) iodine based methods, allowance should be made for the fact that the concentration of $\beta$-limit dextrin in the powder is approximately twice that in other powder preparations (which also contain maltose). A 1% solution of Megazyme $\beta$-limit dextrin has approximately the same concentration of $\beta$-limit dextrin as a 2% solution of lintnerised starch following $\beta$-amylase treatment.

STORAGE CONDITIONS:
Store dry at room temperature in a well sealed container. Under these conditions, the product is stable for several years.

METHOD OF DISSOLUTION:
Accurately weigh 1.00 g of Beta-Limit Dextrin into a 120 mL beaker. Add 100 mL of distilled water or buffer and a magnetic stirrer bar and stir the mixture on a magnetic stirrer for approx. 10 min (until the solution is clear). This solution of $\beta$-limit dextrin can be stored at 4°C for several weeks in a well sealed storage bottle. Prevent microbial contamination by adding a few drops of toluene to the storage bottle.

MEASUREMENT OF $\alpha$-AMYASE USING SCALAR CONTINUOUS FLOW INJECTION ANALYSIS EQUIPMENT:
Details of the recommended procedure are given on the following pages.
MALT, ALPHA-AMYLASE ASSAY PROCEDURE
(based on SKALAR Continuous Flow Analyser Equipment and MEGAZYME Beta-Limit Dextrin)

1 SCOPE
The determination of the $\alpha$-amylase activity of malt measured as the dextrinisation time of a standard beta-limit dextrin solution.

2 FIELD OF APPLICATION
The method can be applied to malts in the range 5-100 DU (dextrinising units).

3 PRINCIPLE
3.1 A saline extract of malt, prepared at 20°C, is allowed to react in the Skalar equipment with a buffered limit dextrin substrate for 10 min at 35°C.

3.2 The amount of $\alpha$-amylase is estimated by spectrophotometric measurement, at 610 nm, of the rate of breakdown of the colour of a beta-limit dextrin/iodine complex.

3.3 The $\alpha$-amylase activity is expressed as the quantity of $\alpha$-amylase which will dextrinise a beta-limit dextrin substrate at the rate of 1 g per h at 20°C.

4 REAGENTS
4.1 Unless otherwise stated, use only reagents of recognised analytical grade and only distilled water or water of equivalent purity. Smaller or larger quantities of reagents may be made up if required.

4.2 Sodium chloride solution, 0.5% w/v. Dissolve 5 g sodium chloride (NaCl) in distilled water and make up to 1.0 L.

4.3 Beta-limit dextrin substrate (for Skalar). Use only beta-limit dextrin from Megazyme International Ireland, Bray Business Park, Bray, Co. Wicklow, Ireland (cat. no. P-BLDX). Dissolve 1 g of beta-limit dextrin in 100 mL of dilution buffer (4.4).

4.4 Dilution buffer (for Skalar). Dissolve 11.0 g sodium chloride (NaCl) and 20.7 g sodium acetate trihydrate ($\text{CH}_3\text{COONa.3H}_2\text{O}$) in 800 mL distilled water. Add 4.5 mL glacial acetic acid ($\text{CH}_3\text{COOH}$), 15 mL 30% Brij 35 (or equivalent) and make up to 5.0 L with distilled water. Mix well.

4.5 $\alpha$-Amylase standard (for Skalar). Dissolve 2.2 g $\alpha$-amylase type VIII-A from barley malt (Sigma cat. no. A-2771) in 100 mL of 0.5% NaCl solution. Take this DU value to be 100 and dilute as appropriate to produce intermediate standards.

4.6 Stock iodine solution. Dissolve 5.08 g pure iodine ($\text{I}_2$) crystals and 20.0 g potassium iodide (KI) in distilled water and make up to 200 mL. Store in a dark bottle.

4.7 Iodine working solution. Prepare immediately before use. Dissolve 3.0 g potassium iodine in 800 mL distilled water. Add 10.0 mL stock iodine solution and make up to 1.0 L with distilled water; mix well and store in a dark bottle.
5 **APPARATUS**

5.1 Skalar continuous flow analyser, fitted with an autosampler device, and set up to analyse a portion of prepared extract for its $\alpha$-amylase content.

5.2 Mill. Buhler Universal Laboratory Disk Mill (type DLFU) set to a gap of 0.2 mm.

5.3 Balance, accuracy of $\pm$ 0.005 g.

5.4 Water bath. A bath sufficiently sensitive to maintain the extract temperature at $20+/- 0.5^\circ C$ and fitted with a mechanical stirring device to ensure an even temperature distribution within the loaded bath.

5.5 Extraction jars, with stopper, nominal capacity 250 mL.

5.6 Filter funnels, suitably sized.

5.7 Filter papers, Ederol 12 ready folded or suitable equivalent, cut to fit funnels.

6 **PREPARATION OF SAMPLES**

Ensure samples for analysis are representative of the bulks from which they have been taken.

7 **PROCEDURE**

7.1 Grind about 14 g malt and mix well.

7.2 Weigh 10.0 +/- 0.1 g grist into an extraction jar, add 200 mL of 0.5% w/v NaCl solution (4.2); swirl to mix ensuring that all grist is properly wetted.

7.3 Incubate the flask and contents in the water bath at $20.0 +/- 0.5^\circ C$ for 120 +/- 5 min; swirl the contents of the flask at 20 min intervals, taking care to ensure that as little grist as possible is left above the solution level.

7.4 Filter the infusion through the cut down fluted filter paper, returning the first 50 mL approximately to the filter.

7.5 Mix the filtrate well by swirling, fill an autosampler sample cup and place in the Skalar sample carousel for analysis.
8 EXPRESSION OF RESULTS

8.1 Calculation of the α-amylase level in the malt, in dextrinising units (D.U.) on an “as is” basis, is performed automatically by the Skalar data acquisition and processing system.

8.2 Results may also be expressed on dry material and can be calculated using the formula:

\[
\text{D.U. (dry)} = \frac{\text{D.U. (as is)} \times 100}{100 - M}
\]

where \( M \) = moisture content (% m/m) of the malt sample.

8.3 Report results as D.U. (as is, or dry) to the nearest whole unit.

NOTE 1 Extracts may be stored in a refrigerator for up to 30 h prior to analysis.

NOTE 2 Cross checks should be made from time to time against the reference method.