SUCRASE (MALTASE) + β-GALACTOSIDASE (Lot 111101b)

E-SUCRBG **03/19**

Sucrase (170 U), α-glucosidase (2600 U) and β-galactosidase (3000 U); freeze dried.

For use in the removal of sucrose, maltose and lactose in dietary fibre determinations. In the Integrated Total Dietary Fibre procedure, in HPLC analysis of non-digestible oligosaccharides using the Waters Sugar-Pak column, the fructosyl-trisaccharide β-D Fruf (2→1)-β-D-Fruf-(2→1)-β-D-Fruf, chromatographs at a similar point to the disaccharides, sucrose, maltose and lactose. Accurate determination of this trisaccharide requires the hydrolysis of these disaccharides. This can be achieved using this enzyme mixture.

PROPERTIES

1. ELECTROPHORETIC PURITY:

This is a mixture of sucrase (maltase; from *Bacillus stearothermophilis*):
- Single major band on SDS-gel electrophoresis (57,750) plus β-galactosidase (from *A. niger*)
- Single band on isoelectric focusing

2. ACTIVITY:

This enzyme mixture gives complete hydrolysis of sucrose, maltose and lactose under the defined assay conditions, with no hydrolysis of the trisaccharide, β-D-Fru*f*-(2→1)-β-D-Fru*f*-(2→1)-β-D-Fru*f*.

3. STORAGE CONDITIONS:

The enzyme is supplied as a lyophilised powder and should be stored below -10°C. On dissolution in buffer or water, the enzyme should be stored in the frozen state. **It is recommended** that all buffers used for dilution contain BSA (0.5 mg/mL).

4. PREPARATION OF ENZYME FOR USE:

Dissolve the contents of one vial in 6 mL of 5 mM sodium acetate buffer (pH 5.0). Transfer aliquots of approx. 2 mL to polypropylene tubes and store below -10°C between use. Can be thawed and re-frozen several times.

INCUBATION CONDITIONS:

To **1 mL** of sugar mixture obtained in the Integrated Total Dietary Fibre procedure [Step I(b)] containing up to 5 mg/mL of sucrose, maltose and/or lactose and fructo-triose,

add:

0.1 mL of sucrase/β-galactosidase enzyme mixture, and incubate at 40°C, for 60 min.

Terminate the reaction by incubating the tube at 100°C for 2 min and centrifuge the suspension in a Microfuge at 12,000 rpm for 5 min.

SAMPLE PREPARATION AND HPLC:

Analyse the supernatant solution by HPLC using a Waters Sugar-Pak^R column as described in the Megazyme kit data booklet for the Integrated Total Dietary Fibre method (**K-INTDF**). Calculate the amount of fructo-triose by reference to the D-sorbitol internal standard. This amount should then be added to the determined amount of non-digestible oligosaccharides [NDO; low molecular weight soluble dietary fibre; Soluble Dietary Fibre soluble in 78% aqueous ethanol (SDFS)].