Oligo-α-1,6-GLUCOSIDASE PLUS β-GALACTOSIDASE (Lot 200601)

E-BGOG **06/20**

Oligo-α-1,6-Glucosidase (2,000 U/mL) and β-Galactosidase (2,000 U/mL); ammonium sulfate suspension

For use in the removal of lactose and isomaltose in dietary fiber determinations. In the Rapid Integrated Total Dietary Fiber procedure (AOAC Method 2017.16), in the analysis of non-digestible oligosaccharides in the SDFS fraction using the TOSOH TSK gel permeation columns, the trisaccharide, fructotriose (β-D-Fru*f* (2-1)-β-D-Fru*f*-(2-1)-β-D-Fru*f*, is poorly separated from lactose and isomaltose, making accurate measurement difficult. Accurate measurement of fructotriose requires the hydrolysis and "removal" of both the lactose and isomaltose and this can be achieved using the described enzyme mixture.

PROPERTIES

1. PURITY:
This product is a mixture of microbial oligo-α-1,6-glucosidase (recombinant, pure; MW = 67.3 KDa) plus *A.niger* β-galactosidase (non-recombinant, electrophoretically pure; MW = 125 Kda).

2. ACTIVITY/SPECIFICITY:
Both enzymes are supplied at a concentration of 2,000 U/mL as an ammonium sulphate suspension (full details of each enzyme are given in Megazyme products cat. no. **E-BGLAN** and **E-OAGUM**). The mixture (**E-BGOG**) gives complete hydrolysis of lactose and isomaltose when used as described in this data sheet, with no hydrolysis of fructotriose or raffinose. Oligo-α-1,6-glucosidase will give significant hydrolysis of other isomalto-oligosaccharides present. The β-galactosidase will also give complete hydrolysis of any β-galacto-oligosaccharides present in the SDFS fraction.

3. STORAGE CONDITIONS:
The enzyme is supplied as an ammonium sulphate solution stabilised with 0.02% w/v sodium azide and should be stored at 4°C.

4. PREPARATION OF ENZYME FOR USE:
Use the enzyme as supplied. Gently swirl the vial to ensure uniform suspension of the preparation.

INCUBATION CONDITIONS:

Prepare and deionise 5 mL of SDFS fraction according to **Step I(b)** of the Rapid Integrated TDF method (**K-RINTDF**). Transfer 2 mL of this solution to a 16 x 120 glass test tube and add 0.1 mL of sodium acetate buffer (0.2 M, pH 4.5) plus 0.1 mL of a suspension of **E-BGOG** (oligo-α-1,6-glucosidase plus β-galactosidase) and incubate at 40°C for 30 min. After 30 min, inactivate and denature the enzymes in the incubation mixture by heating the tubes in a boiling water bath for ~ 3 min. Filter a samples of the solution through a 47 mm, 0.45 µm syringe filter.

HPLC:

Apply the samples to TSKgel® $G2500PW_{XL}$ HPLC columns with in-line deionization in place (Bio-Rad deionizing cartridges). Calculate the amount of SDFS using the appropriate Megazyme *Mega-Calc*™ calculator.

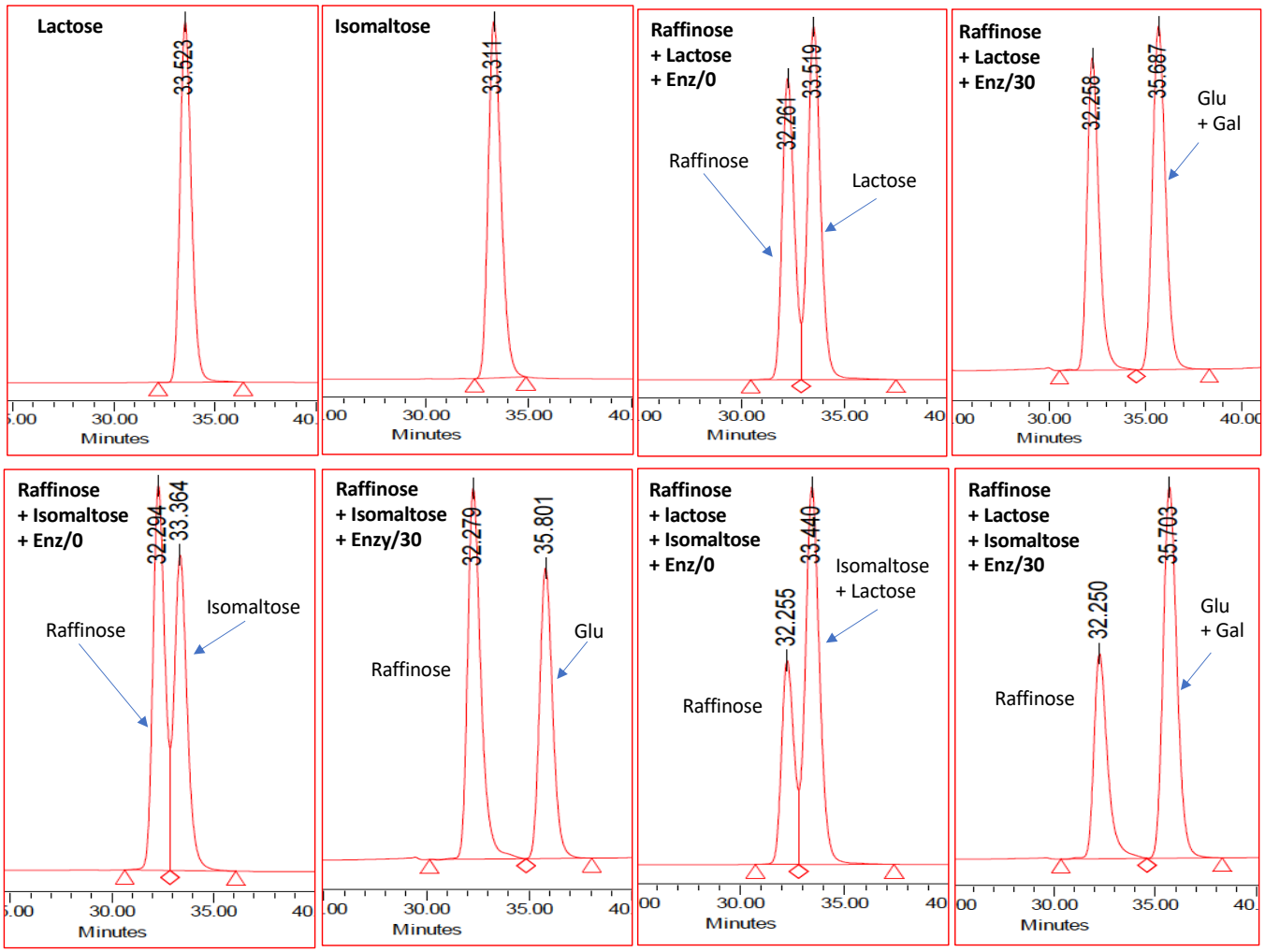

Figure 1. Hydrolysis of lactose and isomaltose by β-galactosidase and oligo-α-1,6-glucosidase (**E-BGOG**) as shown by chromatography on two TSKgel® G2500PW$_{XL}$ HPLC columns in series. Solvent: distilled water, flow rate 0.5 mL/min and temperature 80°C with Bio-Rad deionization column in place. Mixtures of raffinose (~ 5 mg/mL) and lactose (~ 5 mg/mL) and/or isomaltose (5 mg/mL) were chromatographed before and after incubation of 2 mL of the sugar mixtures with a 0.1 mL of a suspension of oligo-α-1,6-glucosidase (2000 U/mL) and β-galactosidase (2,000 U/mL) (Megazyme cat. no. **E-BGOG**) plus 0.1 mL of 0.5 M sodium acetate buffer (pH 4.5) at 40°C for 30 min. The enzyme mixture gives complete hydrolysis of lactose and isomaltose, with no hydrolysis of raffinose or fructotriose (not shown).