

# Megazyme

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## Glucose Oxidase/ Catalase Mixture

FOR THE REMOVAL OF EXCESS  
GLUCOSE IN  
SUCROSE AND FRUCTAN  
DETERMINATIONS

EGOXCA 11/03

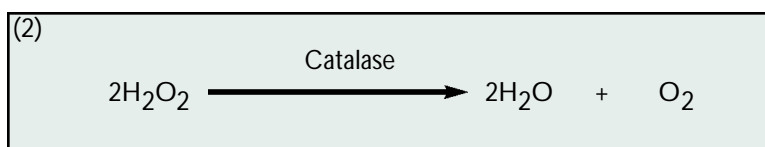
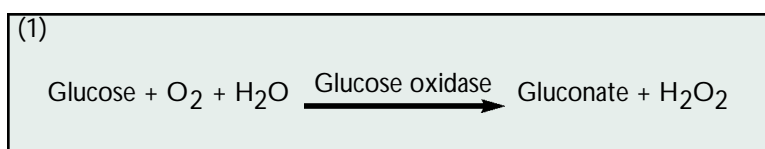


### INTRODUCTION:

This reagent mixture is used to “remove” excess glucose in samples where accurate measurement of low levels of sucrose or fructans is required.

### PRINCIPLE:

The reactions involved are:



### Reagents:

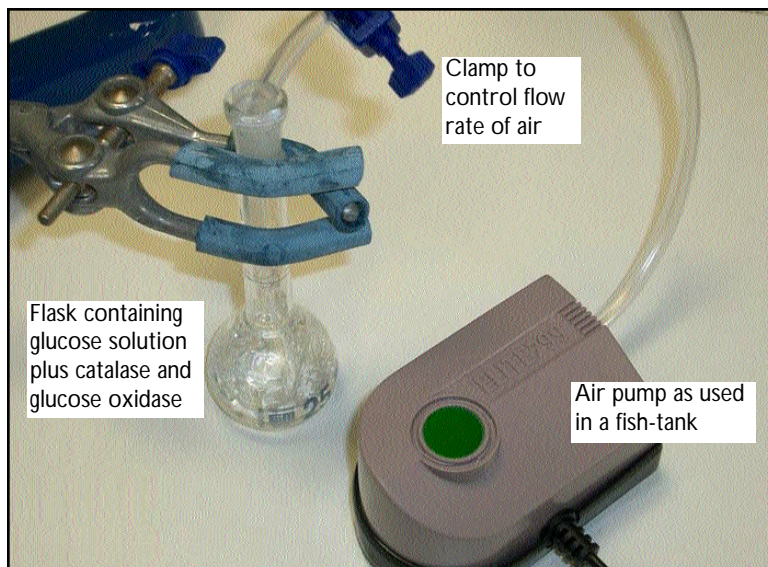
1. **Glucose oxidase (12,000 U) plus Catalase (300,000 U). (Megazyme cat.no. E-GOXCA).**  
Dissolve the contents of 1 vial in 20 ml of 200 mM Tris/HCl buffer (pH 7.6).  
Divide this solution into 2.0 ml aliquots and store at -20°C.
2. Sodium phosphate buffer (300 mM, pH 7.6) containing 5 mM MgCl<sub>2</sub>·7H<sub>2</sub>O.  
Add 53.4 g of di-sodium hydrogen phosphate dihydrate (Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O) to 900 mL of distilled water and dissolve by stirring. Add 1.11 g of MgCl<sub>2</sub>·7H<sub>2</sub>O. Adjust the pH to 7.0 with 1 M sodium hydroxide (40 g/L) and adjust the volume to 1 litre with distilled water. Store at 4°C in a well sealed Duran bottle. To prevent microbial contamination, overlay the solution with 2 drops of toluene.

### Procedure for D-glucose oxidation

Pipette into a 25 ml volumetric flask	
200 mM phosphate buffer solution	5.0 ml
Sample solution (up to approx 0.5% D-glucose)	5.0 ml
Enzyme solution	0.2 ml
Incubate the flask at 25-30°C and pass a current of air (O <sub>2</sub> ) through the mixture for 1 h (see Figure 1). While this oxidation could theoretically lead to a decrease in pH, no significant changes are observed in solutions containing glucose at concentration of up to 5 mg/ml.	

To inactivate the glucose oxidase plus catalase, incubate the volumetric flask in a boiling water bath for 15 min, allow it to cool to room temperature and dilute the contents to the mark with water. Mix and filter, if necessary. Use 0.5 ml of the clear solution for the determination of sucrose. Determine the residual D-glucose in a parallel assay and subtract as usual.

**Figure 1.** Arrangement for the oxidation of glucose by glucose oxidase plus catalase in the presence of a stream of air.





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