

# Enzymatic Ethanol Production Kit Instruction Manual

## 1. Description

12 kinds of enzymes take part in conversion from glucose to ethanol (Fig. 1). This kit is an experimental kit to enable conversion from glucose to ethanol by the continuous enzyme reaction with those 12 kinds of thermostable enzymes *in vitro*.

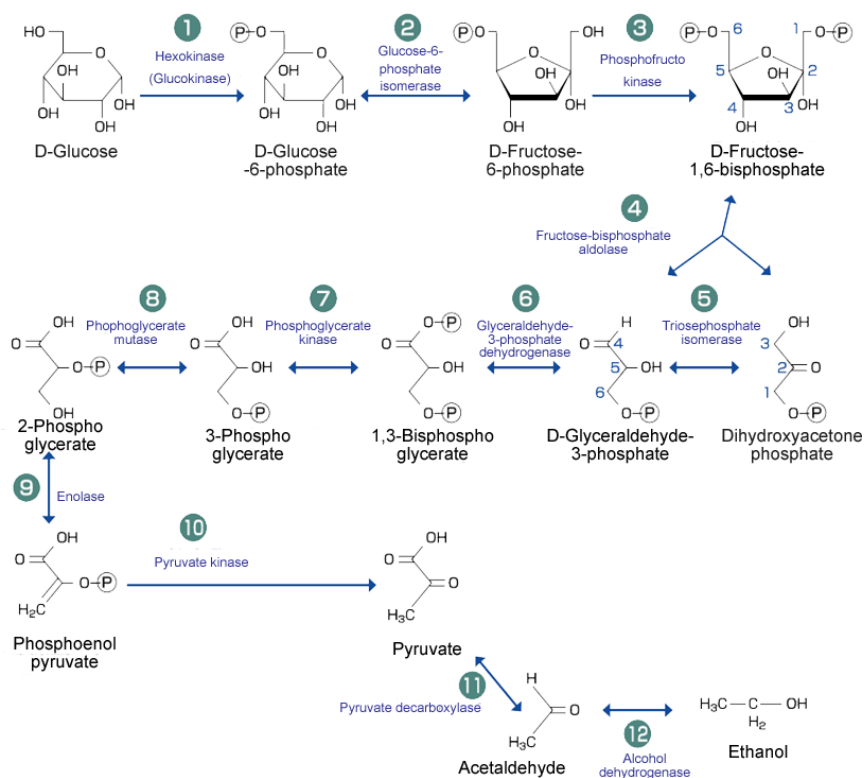


Fig. 1. Scheme of conversion from glucose to ethanol

## 2. Contents

This kit consists of 4 reagents listed below. Enzyme Mix. contains 11 kinds of enzyme 1~11 described in Fig. 1.

Reagents	Volume	Color of tube cap
50 mM Glucose	1 ml	Green
10X Reaction buffer	1 ml	Yellow
Enzyme Mix.	6 ml (1.5 ml X 4)	Blue
Alcohol dehydrogenase	1 ml	Red

## 3. Protocol

### (1) Ethanol production

1. Thaw Enzyme Mix. and Alcohol dehydrogenase on ice.
2. Add 100  $\mu$ l of 10X Reaction buffer, 600  $\mu$ l of Enzyme Mix. and 100  $\mu$ l of Alcohol dehydrogenase to 100  $\mu$ l of sterile deionized water in a sterile 1.5 ml microcentrifuge tube. [Option] Acetaldehyde will be produced if 100  $\mu$ l

of deionized water is added to the reaction instead of Alcohol dehydrogenase.

3. Incubate the reaction mixture at 50 °C.

## (2) Assay for ethanol production

Because the reagents for ethanol detection are not included in this kit, equipment or reagents for ethanol detection are required. We employ the method using alcohol oxidase and peroxidase<sup>1)</sup> to measure the concentration of alcohol (<sup>1)</sup>Gonchar *et.al.*, *Food technol. biotechnol.*, 39(1), 37-42, 2001).

The methods using UV absorption spectrum of NAD<sup>+</sup>/NADH are not suitable to quantify ethanol concentration, since 10X Reaction buffer contains NAD<sup>+</sup>.

## 4. Conversion rate for ethanol production

Fig. 2 shows time course of ethanol production performed as instructed here for 60 minutes.

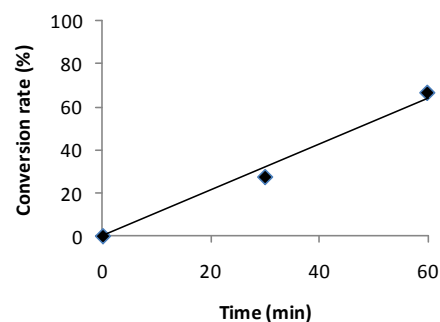


Fig. 2. Conversion kinetics of ethanol

## 5. Precautions

### (1) For protection from hazards

If the reagents come in contact with the mouth, eyes or skin, wash off immediately with a large amount of water. Consult a physician if necessary.

### (2) For assays

This product is for R&D use only.

Avoid repeated freeze-thawing of Enzyme Mix. and Alcohol dehydrogenase.

Aliquots enzymes unless used them at once.

This kit is designed performed at 50 °C. The yield of the product might decrease if performed at temperatures other than 50 °C.

### (3) For disposal

When discarding the reagents, dispose of them according to local or national regulations.

## 6. Storage condition and shelf life

(1) Store at -20 °C

(2) Shelf life after product verification: 12 months

Contact:

Thermostable Enzyme Laboratory Co., Ltd.

5-5-2-501, Minatojima-Minamimachi, Chuo-ku, Kobe, 650-0047

TEL : 078-302-5502 FAX : 078-302-5512

E-mail : info@tainetsu.com



株式会社 耐熱性酵素研究所  
Thermostable Enzyme Laboratory Co., Ltd.