上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

Galactose Oxidase Assay

Method

The reaction velocity is measured in a peroxidase/o-tolidine coupled system as an increase in A425 resulting from the oxidation of galactose. One unit results in a change in A425 of 1.0 per minute at 25° C and pH 6.0 under the defined conditions.

Reagents

1）0.1 M Potassium phosphate buffer, pH 6.0

2）0.5% o-tolidine. Note: o-tolidine has been reported to be carcinogenic. Handle with care.

3）Peroxidase. Dissolve at a concentration of approximately 60 u/ml in reagent grade water.

4）10% galactose. Allow to come to equilibrium of mutarotation by allowing to stand overnight.

Enzyme

Dissolve at a concentration of 1 mg/ml in reagent grade water. Dilute further for assay to a concentration of 0.2 - 0.5 units/ml.

Procedure

1）Adjust spectrophotometer to 425 nm and 25° C.

2）Prepare tolidine-buffer mixture by adding 0.1 ml tolidine to 12 ml 0.1 M potassium phosphate buffer pH 6.0.

3）Pipette into each cuvette as follows:

Tolidine-buffer solution	1.7 ml
10% Galactose	1.5 ml
Peroxidase	0.1 ml

Incubate in spectrophotometer at 25° C for 3 - 4 mintues to achieve temperature equilibration and establish blank rate, if any. Add 0.1 ml of appropriately diluted enzyme and record increase in A425/min. from initial linear portion of the curve.

上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

Calculation

$$\frac{\text{Units}}{\text{mg}} = \frac{\frac{\Delta A_{425}}{\text{min}}}{\text{mg enzyme in reaction mixture}}$$