

# L-Galactose-1,4-Lactone Dehydrogenase (Gal LDH) Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer

**Cat No:** BC1250

**Size:**50T/48S

## **Components:**

Extract solution: 60 mL×1. Storage at 4°C.

Reagent I: Powder×1. Storage at -20°C and avoid light. Dissolve thoroughly with 40 mL distilled water before use.

Reagent II: Powder×1. Storage at 4°C and avoid light. Dissolve thoroughly with 5 mL distilled water before use.

## **Product Description:**

L-galactose way is the key for the synthesis of AsA. Gal LDH is located in the mitochondrial inner membrane and is responsible for the final step of catalyzing the biosynthesis of AsA in plants. Gal LDH is one of the key enzymes in this pathway. It plays an important role in the accumulation of AsA in plants.

Gal LDH catalyzes the reduction of L-galactonolactone to oxidized cytochrome c (Cyt c). Reduced Cyt c has an absorption peak at 550 nm. Measure the increasing rate of reduced Cyt c can calculate the activity of Gal LDH.

## **Reagents and Equipment Required but Not Provided:**

Spectrophotometer, desk centrifuge, adjustable pipette, 1 mL glass cuvette, mortar/homogenizer, ice and distilled water.

## **Procedure:**

### **I. Sample extraction**

Add 1 mL of Extract solution into 0.1 g of sample, fully grind on ice. Centrifuge at 13000 g and 4°C for 10 min. Supernatant on ice is ready for test.

### **II. Determination procedure**

1. Preheat spectrophotometer for 30 min, adjust the wavelength to 550 nm, set zero with distilled water.
2. According to the sample amount, take Reagent I at 25°C in water bath for 30 min. The rest store at -20°C.
3. Blank tube: Add 100  $\mu$ L of distilled water, 800  $\mu$ L of Reagent I and 100  $\mu$ L of Reagent II in 1 mL glass cuvette, mix thoroughly, detect absorbance for 10s and 130s at 550 nm, record the absorbance A1 at 10s and A2 at 130s.  $\Delta A(B) = A2 - A1$ .
4. Test tube: Add 100  $\mu$ L of supernatant, 800  $\mu$ L of Reagent I and 100  $\mu$ L of Reagent II in 1 mL glass cuvette, mix thoroughly and quickly, detect absorbance for 10s and 130s at 550 nm, record the absorbance

A3 at 10s and A4 at 130s.  $\Delta A(T) = A4 - A3$ .

### III. Calculation:

#### 1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the reduction of 1  $\mu\text{mol}$  Cyt c at 25°C per minute every milligram protein.

$$\begin{aligned}\text{Gal LDH (U/mg prot)} &= [\Delta A(T) - \Delta A(B)] \div (\epsilon \times d) \times V_{rv} \times 10^6 \div (V_s \times C_{pr}) \div T \\ &= 0.289 \times [\Delta A(T) - \Delta A(B)] \div C_{pr}\end{aligned}$$

#### 2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the reduction of 1  $\mu\text{mol}$  Cyt c at 25°C per minute every gram sample.

$$\begin{aligned}\text{Gal LDH (U/g)} &= [\Delta A(T) - \Delta A(B)] \div (\epsilon \times d) \times V_{rv} \times 10^6 \div (V_s \div V_{sv} \times W) \div T \\ &= 0.289 \times [\Delta A(T) - \Delta A(B)] \div W\end{aligned}$$

$\epsilon$ : Reduced Cyt c molar extinction coefficient,  $17.3 \times 10^6$  L/mol/cm;

d: Light path of cuvette, 1 cm;

$V_{rv}$ : Total reaction volume, 1 mL = 0.001 L;

$10^6$ : 1 mol =  $1 \times 10^6$   $\mu\text{mol}$ ;

$V_s$ : Supernate volume, 100  $\mu\text{l}$  = 0.1 mL;

$V_{sv}$ : Extraction volume, 1 mL;

$C_{pr}$ : Supernatant protein concentration (mg/mL); need to detect separately, suggest use PC0020, BCA Protein Assay Kit;

T: Reaction time, 2 min;

W: Sample weight, g;

#### Note:

1. Note the temperature when detecting enzyme activity suggest that take a beaker with 37°C distilled water, put the cuvette and react solution in 37°C beaker.
2. Advice that two person conduct the experiment, One person measures absorbance and the other one record data time to make accuracy of the experimental results.
3. After adding the last reagent, mix thoroughly and quickly and detect the OD value.

#### Examples:

1. Add 0.1g kiwi fruit to 1mL extract solution and grind thoroughly on ice, centrifuge with 13000g at 4°C for 10min, take supernatant on ice, follow the determination procedure to operate, and calculate  $\Delta A(T) = A4 - A3 = 0.881 - 0.784 = 0.097$ ,  $\Delta A(B) = A2 - A1 = 0$ , according with mass of sample to calculate enzyme activity: Gal LDH (U/g weight) =  $0.289 \times (\Delta A(T) - \Delta A(B)) \div W = 0.2803$  U/g weight.

#### Related Products:

BC1230/BC1235	Ascorbic Acid (AsA) Content Assay Kit
BC1240/BC1245	Dehydroascorbic Acid (DHA) Assay Kit
BC0650/BC0655	Monodehydroascorbate Reductase(MDHAR) Activity Assay Kit