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

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

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC1255

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 110 mL×1	2-8℃
Reagent I	Powder×1	-20℃
Reagent II	Powder×1	2-8℃

Solution Preparation:

1. Reagent I: Dissolve thoroughly with 16 mL distilled water before use.
2. Reagent II: Dissolve thoroughly with 2 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/microplate reader, micro glass cuvette/96 well flat-bottom plate, desk centrifuge, adjustable pipette, 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℃ for 10 min. Supernatant on ice is ready for test.

II、Determination procedure

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 550 nm, set zero with distilled water.
2. According to the sample amount, take Reagent I into a 25℃water bath for 30 min. The rest store at -20℃.
3. Blank tube: Add 20 μL of distilled water, 160 μL of Reagent I and 20 μL of Reagent II in micro glass

cuvette/96 well flat-bottom plate, mix thoroughly and quickly, detect absorbance for 10s and 130s at 550 nm, record the absorbance A1 at 10s and A2 at 130s. ΔA(B)= A2-A1.

4. Test tube: Add 20 μL of supernatant, 160 μL of Reagent I and 20 μL of Reagent II in micro glass cuvette/96 well flat-bottom plate, mix thoroughly and quickly, detect absorbance for 10s and 130s at 550 nm, record the absorbance A3 at 10s and A4 at 130s.ΔA(T)= A4-A3.

III. Calculation:

a. Micro glass cuvette:

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the reduction of 1 μmol Cyt c at 25℃ per minute every milligram protein.

Gal LDH (U/mg prot) = [ΔA(T)-ΔA(B)] ÷(ε×d) ×Vrv×10^6÷(Vs×Cpr) ÷T

=0.289×[ΔA(T)-ΔA(B)] ÷Cpr

2. Sample weight:

One unit of enzyme activity is defined as the amount of enzyme catalyzes the reduction of 1 μmol Cyt c at 25℃ per minute every gram sample.

Gal LDH (U/g weight) =[ΔA(T)-ΔA(B)] ÷(ε×d) ×Vrv×106÷(Vs÷Vsv×W) ÷T

=0.289×[ΔA(T)-ΔA(B)] ÷W

ε: Reduced Cyt c umolar extinction coefficient,17.3×10^3 L/mol/cm;

d: Light path of cuvette, 1 cm;

Vrv: Reaction total volume, 0.2 mL=0.0002 L;

10^6:1 mol=1×10^6 μmol;

Vs: Supernate volume, 20 μL=0.02 mL;

Vsv: Extraction volume, 1 mL;

Cpr: Supernatant protein concentration (mg/mL); need to detect separately, suggest use PC0020, BCA Protein Assay Kit;

W: Sample weight, g;

T: Reaction time, 2 min.

b. 96 well flat-bottom plate

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the reduction of 1 μmol Cyt c at 25℃ per minute every milligram protein.

Gal LDH (U/mg prot) = [ΔA(T)-ΔA(B)] ÷(ε×d) ×Vrv×10^6÷(Vs×Cpr) ÷T

=0.482×[ΔA(T)-ΔA(B)] ÷Cpr

2. Sample weight:

One unit of enzyme activity is defined as the amount of enzyme catalyzes the reduction of 1 μmol Cyt c at 25℃ per minute every gram sample.

Gal LDH (U/g weight) =[ΔA(T)-ΔA(B)] ÷(ε×d) ×Vrv×106÷(Vs÷Vsv×W)÷T

$=0.482\times[\Delta A(T)-\Delta A(B)]\div W$

ε: Reduced Cyt c umolar extinction coefficient, 17.3×10^3 L/mol/cm;
d: Light path of 96 well flat-bottom plate, 0.6 cm;
Vrv: Reaction total volume, 0.2 mL=0.0002 L;
10^6:1 mol=1×10^6 μmol;
Vs: Supernate volume, 20 μL=0.02 mL;
Vsv: Extraction volume, 1 mL;
Cpr: Supernatant protein concentration (mg/mL); need to detect separately, suggest use PC0020, BCA Protein Assay Kit;
W: Sample weight, g;
T: Reaction time, 2 min.

Note:

1. Note the temperature when detecting enzyme activity, advice that take a beaker with 37℃ distilled water, cuvette and reactive solution, put the beaker in 37℃ waterbath. If use the 96 well flat-bottom plate, it should be placed in a 37℃ incubator during the reaction.
2. Advice that two people do the experiment, one does colorimetric and the other record time to make accuracy of the experimental results.
3. Mix thoroughly and fast after add the last reagent, detect the OD value.

Examples:

1. Add 0.1g potato to 1mL extract solution and grind thoroughly on ice, centrifuge with 13000g at 4℃ for 10min, take supernatant on ice, follow the determination procedure to operate, and calculate ΔA (T)=A4-A3=0.9253-0.7844=0.1409, ΔA(B)=A2-A1=0, according with mass of sample to calculate enzyme activity: Gal LDH（U/g weight）=0.289×(ΔA (T)-ΔA (B))÷W=0.4072 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