L-galactono-1,4-lactone Dehydrogenase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1050

(Version 1.2C)

Detection and Quantification of L-galactono-1,4-lactone Dehydrogenase(GalLDH) Activity in Tissue extracts, Cell lysateSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

L-galactono-1,4-lactone Dehydrogenase (EC 1.3.2.3) catalyzes the last step in the main pathway of vitamin C (L-ascorbic acid) biosynthesis in higher plants. L-galactono-1,4-lactone Dehydrogenase ActivityColorimetric Microplate Assay Kit provides a simple and direct procedure for measuring L-galactono-1,4-lactone Dehydrogenaseactivity levels.The enzyme catalysed reaction products reducedCyt c can be measured at a colorimetric readout at 550 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Substrate I	Powder x 1	4 °C, keep in dark
Substrate II	Powder x 1	4 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Substrate I: Add 17 ml distilled waterto dissolve before use.Keep in dark and store at 4 °C for 1-2 weeks or -20°C for 3-4months after reconstitution.

Substrate II: Briefly centrifuge prior to opening.Add 1 ml distilled waterto dissolve before use. Prepare for immediate use or store at -20°C for 3-4 daysafter reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay Buffer for 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 13000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice, centrifuged at 13000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. ASSAY PROCEDURE

Warm theSubstrateI and Substrate II to room temperature before use.

Add following reagents in the microplate:

Reagent*	Sample**	Blank
Sample	20 µl	--
Distilled water	--	20 µl
SubstrateI	170 µl	170 µl
SubstrateII	10 µl	10 µl
Mix,measured at 550 nm and recordthe absorbance of 10th second and 130th second.		

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VI. CALCULATION

Unit Definition: One unit of GalLDH is the amount of enzyme that will reduce 1μmolCytc per minute.

1.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(OD_{Sample(130)}-OD_{Sample(10)}) - (OD_{Control(130)}-OD_{Control(10)})}{\varepsilon \times d \times C_{Protein} \times T} \times \frac{V_{Total}}{V_{Sample}} (U/mg)$$

1.2 According to the weight of sample

$$\text{Activity} = \frac{(OD_{Sample(130)}-OD_{Sample(10)}) - (OD_{Control(130)}-OD_{Control(10)})}{\varepsilon \times d \times W \times T} \times \frac{V_{Total} \times V_{Assay}}{V_{Sample}} (U/g)$$

1.3According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(OD_{Sample(130)}-OD_{Sample(10)}) - (OD_{Control(130)}-OD_{Control(10)})}{\varepsilon \times d \times N \times T} \times \frac{V_{Total} \times V_{Assay}}{V_{Sample}} (U/10^4)$$

1.4 According to the volume of sample

$$\text{Activity} = \frac{(OD_{Sample(130)}-OD_{Sample(10)}) - (OD_{Control(130)}-OD_{Control(10)})}{\varepsilon \times d \times T} \times \frac{V_{Total}}{V_{Sample}} (U/mg)$$

ε: molar extinction coefficient, 17.3×10^3L/mol/cm = 17.3 ml/μmol/cm

d: the optical path of 96-Well microplate, 0.6 cm;

$C_{Protein}$: the protein concentration, mg/ml

W: the weight of sample, g

N: the quantity of cell or bacteria, N $\times 10^4$

V_{Total}: thetotal volume of all reagents in assay procedure, ml

V_{Sample}: the volume of sample in assay procedure, ml

V_{Assay}: the volume of Assay Buffer II in sample preparation, ml

T: the reaction time, minutes