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

Instructions for Use | Cat. No.: ARS6657

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

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

1. 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 Dehydrogenase activity levels.The enzyme catalysed reaction products reducedCyt c can be measured at a colorimetric readout at 550 nm.

2. 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	-

3. 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

4. Reagent Preparation

Substrate I: Add 17 mL distilled water to 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 water to 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.

5. Sample Preparation

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

2. For tissue samples Weigh out 0.1 g tissue, homogenize with 1 mL Assay Buffer on ice, centrifuged at 13000 g 4°C for 10 minutes , take the supernatant into a new centrifuge tube and keep it on ice for detection.

6. Assay Procedure

Warm theSubstrate I 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 of10th second and130th second.		

Note:

*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.

7. Calculation

Unit Definition:

One unit of Gal LDH is the amount of enzyme that will reduce 1μmol Cytc per minute .

1.1 According to the protein concentration of sample

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

1.2 According to the weight of sample

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

1.3According to the quantity of cells or bacteria

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

1.4 According to the volume of sample

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

Item	Definition
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 × 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