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

Catalog # a.{унпонмо

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

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.
The enzyme catalysed reaction products reduced Cyt 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 ml x 4	4 °C
Substrate I	Powder x 1	4 °C, keep in dark
Substrate II	Powder x 1	4 °C
Technical Manual	1 Manual	

Note:

Substrate I: add 17 ml distilled water to dissolve before use.

Substrate II: add 1 ml distilled water to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

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

IV. 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%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 13000g 4 °C for 10 minutes, 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 13000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Warm the Substrate 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
Substrate I	170 µl	170 µl
Substrate II	10 µl	10 µl
Mix, measured at 550 nm and record the absorbance of 10th second and 130th second.		

VI. CALCULATION

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

1. According to the protein concentration of sample

$$GalLDH\ (U/mg) = [(OD_{Sample(130S)} - OD_{Sample(10S)}) - (OD_{Blank(130S)} - OD_{Blank\ (10S)})] / (\varepsilon \times d) \times V_{Total} \times 10^6 / (V_{Sample} \times C_{Protein}) / T$$

$$= 481.7 \times [(OD_{Sample(130S)} - OD_{Sample(10S)}) - (OD_{Blank(130S)} - OD_{Blank\ (10S)})] / C_{Protein}$$

2. According to the weight of sample

$$GalLDH\ (U/g) = [(OD_{Sample(130S)} - OD_{Sample(10S)}) - (OD_{Blank(130S)} - OD_{Blank\ (10S)})] / (\varepsilon \times d) \times V_{Total} \times 10^6 / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 481.7 \times [(OD_{Sample(130S)} - OD_{Sample(10S)}) - (OD_{Blank(130S)} - OD_{Blank\ (10S)})] / W$$

3. According to the quantity of cells or bacteria

$$GalLDH\ (U/10^4) = [(OD_{Sample(130S)} - OD_{Sample(10S)}) - (OD_{Blank(130S)} - OD_{Blank\ (10S)})] / (\varepsilon \times d) \times V_{Total} \times 10^6 / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 481.7 \times [(OD_{Sample(130S)} - OD_{Sample(10S)}) / (OD_{Standard(130S)} - OD_{Standard\ (10S)})] / N$$

ε: molar extinction coefficient, 17.3×10^3 L/mol/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}: the total volume of the enzymatic reaction, 0.2 ml;

V_{Sample}: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 2 minutes.