Beta-Galactosidase Microplate Assay Kit

Catalog # AS0099

Detection and Quantification of Beta-Galactosidase Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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I. INTRODUCTION

Beta galactosidase is a hydrolase enzyme that cleaves beta-linked terminal galactosyl residues from gangliosides, glycoproteins, and glycosaminoglycans. Beta galactosidase is an essential enzyme in the human body. Deficiencies in the protein can result in galactosialidosis or Morquio B syndrome. Senescent cells display senescence-associated expression of beta galactosidase activity.

The assay is initiated with the enzymatic hydrolysis of the glucoside byβ-Galactosidase. The enzyme catalysed reaction productsp-nitrophenol, can be measured at a colorimetric readout at 405 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	5 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Stop Solution	15 ml x 1	4 °C
Standard (1mmol/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: Add 2 ml Reaction Bufferto dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 405 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 mlAssay buffer for 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s,repeat 30 times); centrifuged at 10,000g 4°C for 20 minutes, 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 10,000g 4°C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Control	Standard	Blank
Sample	10 µl	--	--	--
Distilled water	--	10 µl	--	--
Substrate	20 µl	20 µl	--	--
Reaction Buffer	20 µl	20 µl	--	--
Mix, put it in the oven, 37°C for 30 minutes.				
Standard	--	--	50 µl	--
Stop Solution	150 µl	150 µl	150 µl	200 µl
Mix, record absorbance measured at 405 nm.				

VI. CALCULATION

Unit Definition: One unit ofβ-Galactosidase activity is defined asthe enzyme thatgenerates 1μmol of p-nitrophenol per hour.

1. According to the protein concentration of sample

$$\beta\text{-GAL (U/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (C_{Protein} \times V_{Sample}) / T$$

$$= 10 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\beta\text{-GAL (U/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 10 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3.According to the quantity of cells or bacteria

$$\beta\text{-GAL (U/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 10 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

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

$C_{Standard}$: the concentration of Standard, 1mmol/L = 1μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N ×10^4;

$V_{Standard}$: the volume of standard, 0.05 ml;

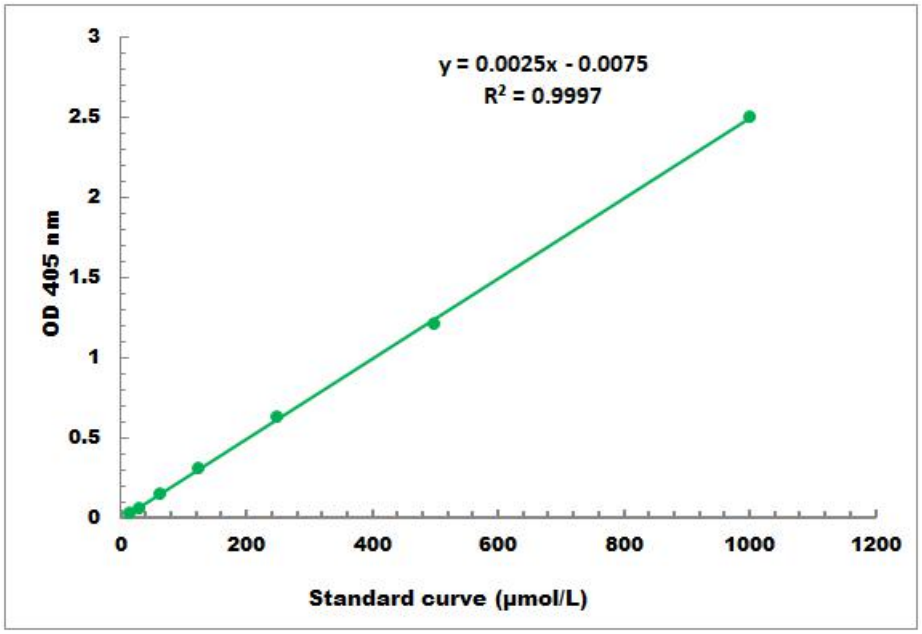
V_{Sample}: the volume of sample, 0.01 ml;

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

T: the reaction time, 0.5 hour.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 10μmol/L -1000μmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES