



Beta-Galactosidase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1099

(Version 1.4H)

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

For research use only. Not for diagnostic or therapeutic 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 by β -Galactosidase. The enzyme catalysed reaction products p-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
Dye Reagent	15 ml x 1	4 °C
Standard (1mmol/L)	1 ml x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 1000/500/250/125/62.5/31.25/15.625 $\mu\text{mol/L}$.

Substrate: Briefly centrifuge prior to opening. Add 2 ml Reaction Buffer to dissolve before use. Store at 4°C for 1-2 weeks or -20 °C for 3 months.

Positive Control: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate stock solution. Dilute the stock solution 100-fold using Assay Buffer to prepare the Positive Control working solution (eg. 10 μl to 990 μl distilled water). Store at -80 °C for 1 month.

V. 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 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

Weigh out 0.1 g tissue, homogenize with 1 ml Assay 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.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Sample	10 μ l	--	--	--	--
Distilled water	--	10 μ l	--	50 μ l	--
Positive Control	--	--	--	--	10 μ l
Substrate	20 μ l	20 μ l	--	--	20 μ l
Reaction Buffer	20 μ l	20 μ l	--	--	20 μ l
Mix, put it in the oven, 37°C for 30 minutes.					
Standard	--	--	50 μ l	--	--
Dye Reagent	150 μ l	150 μ l	150 μ l	150 μ l	150 μ l
Mix, record absorbance measured at 405 nm.					

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.

**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.

VII. CALCULATION

Unit Definition: One unit of β -Galactosidase activity is defined as the enzyme generates 1 μ mol of p-nitrophenol per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{\text{Standard}}}{V_{\text{Sample}}} \times n (\text{U/mL})$$

2. According to one point of the standard OD value and concentration

2.1 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

C_{Standard} : the concentration of standard, $\mu\text{mol/mL}$

V_{Standard} : the volume of standard in assay procedure, mL

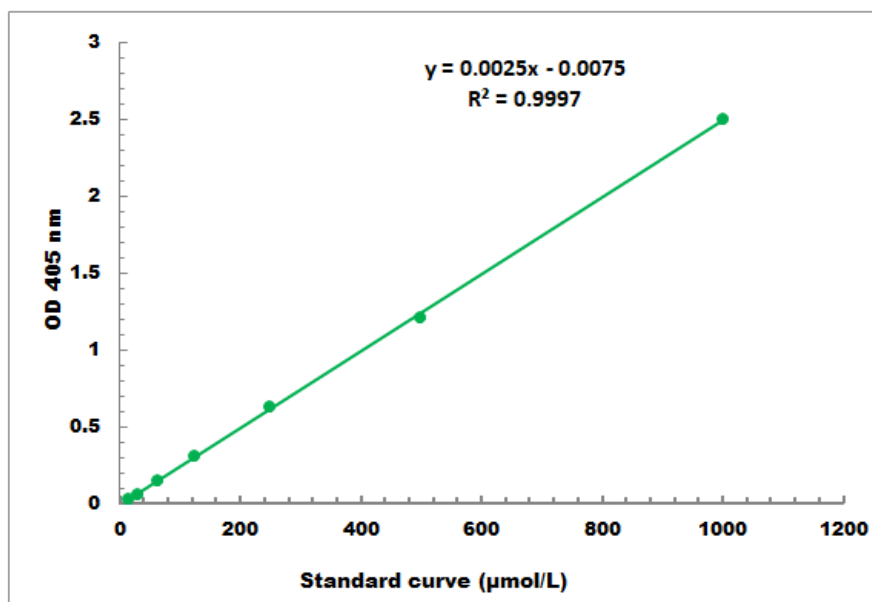
V_{Sample} : the volume of sample in assay procedure, mL

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

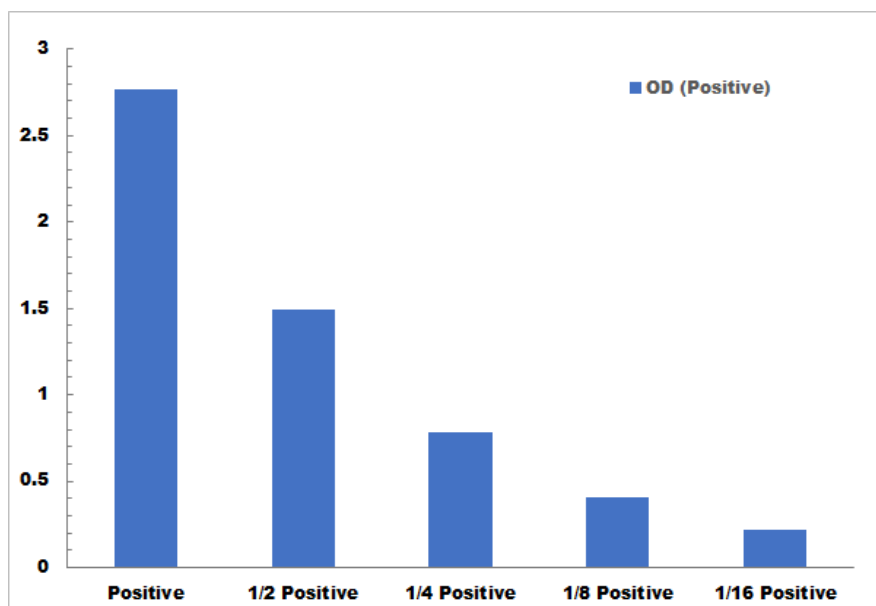
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 10μmol/L -1000μmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration