G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Fluorescent β-Galactosidase Assay (MUG)

(Cat. #786-654)

INTRODUCTION

The Fluorescent β-Galactosidase Assay (MUG) is a highly sensitive, fluorescent assay for determining the β-galactosidase activity in the lysates of cells transfected with a β-galactosidase expression construct

The study of the lac operon has played an important role in understanding the control of gene expression in bacteria. In prokaryotes, gene expression is controlled primarily at the level of transcription. For eukaryotes, the promoter activity can be analyzed by using fusion genes containing the promoter of interest attached to the bacterial β-galactosidase gene and be assayed by measuring β-galactosidase activity. Because β-galactosidase has a high turnover rate and is absent in mammalian cells it serves as a very useful and sensitive reporting tool for gene expression.

Esters of the fluorescent compound, 4-methylumbelliferone (4-MU), provide a sensitive, quantitative assay for β-galactosidase. 4-methylumbelliferyl-β-D-galactopyranoside (4-MUG) is a substrate of β-galactosidase that does not fluoresce until cleaved by the enzyme to generate the fluorophore 4-methylumbelliferone. The assay can be used with extracts from different expression systems including mammalian, insect cells, yeast, and bacteria.

The Fluorescent β-Galactosidase Assay (MUG) provides a 96 well assay format for galactosidase activity that is suitable for high throughput applications. The production of the fluorphore is monitored at an emission/excitation wavelength of 365/460nm. This kit is designed for 500 micro-well assays.

ITEM(S) SUPPLIED (Cat. # 786-654)

Description	Size
Mammalian Cell *PE LB*™	100ml
Fluorescent β-Galactosidase Assay Buffer [2X]	60ml
Fluorescent β-Galactosidase Assay Stop Solution [5X]	15ml
4-MUG (4-Methylumbelliferyl-β-galactopyranoside)	5 x 750µl
Fluorescent β-Galactosidase Assay Standard (4-Methylumbelliferone[1mM])	100µl

STORAGE CONDITIONS

The 4-MUG Substrate and Fluorescent β-Galactosidase Assay Standard should be stored at -20°C and all other components at 4°C. If stored correctly the kit is stable for 1 year from the date of purchase.

ADDITIONAL MATERIALS REQUIRED

- Phosphate-buffered saline (PBS)
- 96-well plate for fluorescent reader
- Additional Mammalian Cell *PE LB*™ (Cat. # 786-180) may be purchased if required.

PROTOCOLS

1. Harvest Adherent Cells

1. Use the information in Table 1 to determine the correct volumes of wash and lysis solutions you require at each step.
2. Remove the culture medium from the adherent transfected cells.
   NOTE: *We recommend a control sample is prepared for endogenous β-galactosidase by using an equivalent plate/well of mock transfected cells.*
3. Wash the cells once with 1X PBS. Remove the PBS wash.

Tissue Culture Plate Format	1X PBS Wash Required (ml)	Mammalian Cell *PE LB*™ Required (µl)
96-well	0.1	10
48-well	0.25	20
24-well	0.5	50
12-well	1	100
6-well	2.5	250
35mm dish	2.5	250
60mm dish	5	500
100mm dish	10	1000
150mm dish	25	2500

Table 1: Volumes of PBS wash and Mammalian PELB™ required.

4. Add the indicated volume of the Mammalian Cell-*PE LB*™ (Table 1).
5. Freeze the plates/dishes at -20°C for 30 minutes for complete lysis and then thaw at room temperature.
   NOTE: *View the plates under a light microscope to check for complete lysis, if inadequate repeat the freeze-thaw cycle.*
6. The lysates are transferred to a centrifuge tube and clarified by centrifugation at 12,000x g for 5 minutes at 4°C.
7. Transfer the supernatants to a fresh tube and store at -20°C or proceed to the β-Galactosidase Assays.

2. Harvest Cells in Suspension

1. Transfer the transfected cells to a suitable centrifuge tube and centrifuge at 200 x g for 5 minutes to pellet the cells. Remove the supernatant.
   NOTE: *We recommend a control sample is prepared for endogenous β-galactosidase by using an equivalent number of mock transfected cells.*
2. Gently, wash the cells once with 5ml 1X PBS. Remove the PBS wash by centrifuging at 200 x g for 5 minutes to pellet the cells. Remove the supernatant.
3. Add 500µl Mammalian *PE LB*™ to each cell pellet and vortex for 10 seconds.
4. Freeze the cells for 30 minutes at -20°C and thaw at room temperature.
5. The lysates are transferred to a centrifuge tube and clarified by centrifugation at 12,000x g for 5 minutes at 4°C.
6. Transfer the supernatants to a fresh tube and store at -20°C or proceed to the β-Galactosidase Assays.

3. Fluorescent β-Galactosidase Assay

The following procedure describes a fluorescent assay for measuring β-galactosidase activity in mammalian cells. Modification of the procedure may be required in cases of extremely high or low expression levels, when using extracts derived from other organisms or when using other plate formats. This is a 96 well format. Designed for 5µl lysate, which may be adjusted to a 50µl maximum volume of lysate.

Thaw all kit components at room temperature and gently swirl to ensure proper mixing. Place the 4-MUG on ice after thawing.

1. If frozen, allow the cell lysates to thaw at room temperature.
2. For a 96-well plate, prepare 15ml 1X Fluorescent β-Galactosidase Assay Buffer by mixing 7.5ml Fluorescent β-Galactosidase Assay Buffer [2X] with 7.5ml DI water.
3. Prepare the Assay Reaction Mix by adding 750µl 4-MUG solution to the 15ml 1X Fluorescent β-Galactosidase Assay Buffer and vortex to mix.
4. For a 96-well plate, prepare 10ml 1X Stop Solution by mixing 2ml Fluorescent β-Galactosidase Assay Stop Solution [5X]with 8ml DI water.

Prepare 4-MU Standards

1. Dilute the Fluorescent β-Galactosidase Assay Standard to 10µM by adding 20µl to 2ml pure water.
2. Transfer 50µl of the 10µM Fluorescent β-Galactosidase Assay Standard to 5ml 1X Stop Solution for a 100nM Fluorescent β-Galactosidase Assay Standard.
3. Dilute the 100nM Fluorescent β-Galactosidase Assay Standard as indicated below:
   ***NOTE**: The prepared Fluorescent β-Galactosidase Assay Standards can be stored at -20°C for up to 1 month. Do not freeze thaw the standards.*

Tube	100nM Fluorescent β-Galactosidase Assay Standard (µl)	Stop Solution [1X] (µl)	[4-MU] (nM)
1	0	1000	0
2	100	900	10
3	200	800	20
4	400	600	40
5	600	400	60
6	800	200	80
7	1000	0	100

5. Add 5µl clarified cell lysates to the wells of a 96-well plate. We recommend performing the assay in triplicate. Add 5µl Mammalian *PE LB*™ to three wells that will act as your blank.
6. Add 145µl Assay Reaction Mix (step 3) to the wells and mix by pipetting up and down.
7. Cover the plate and protect from light with aluminum foil.
8. Incubate at 37°C for 15-60 minutes.
9. Add 75µl Stop Solution, mix well and remove all air bubbles prior to reading.
10. Add 200µl 4-MU standards to the plate in duplicate.
11. Measure fluorescent at 360nm excitation and 440nm emission.
12. Calculate the average relative fluorescence units (RFU) for samples and standards performed in triplicate or duplicate.
13. Subtract the mock-transfected cell lysate RFU from the readings to control for endogenous β-galactosidase.
14. Plot a calibration curve of Fluorescent β-Galactosidase Assay Standard (4-MU) concentrations against the average relative fluorescence units (RFU).
15. To calculate the concentration of 4-MU substrate released, plot the best fit linear line and use the equation of the line to calculate 4-MU formed.
    a. The equation for a line is y = mx + b, where y is the RFU; x is the sample concentration (nM), m is the slope of the line and b is the y-intercept

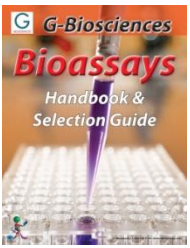
b. Determine the amount of MUG hydrolyzed in the sample by inserting the RFU values (y-value) into the following equation: [4-MU formed] (nM)= (RFU value-b)/m

TROUBLESHOOTING

Observation	Suggestion
No fluorescence	1. Cell lysis is incomplete. Repeat the freeze–thaw cycle or supplement the Mammalian Cell *PE LB*™ with Triton® X-100 to a 1% concentration. 2. Cells are not efficiently transfected with the reporter plasmid. Optimize the transfection conditions. 3. Stain cells for β-galactosidase activity in situ to determine transfection efficiency. 4. Verify that the assay incubation temperature was 37°C. 5. Cell lysate contains a low β-galactosidase concentration Incubate the sample for a longer time (up to 24 hours) at 37°C.
Fluorescence is too intense	1. Cell lysate contains a high β-galactosidase concentration. Decrease the assay incubation time. 2. Decrease the β-galactosidase concentration by using less cell lysate in the assay and diluting the cell lysate with Mammalian Cell *PE LB*™ before performing the assay.

RELATED PRODUCTS

Download our Bioassays Handbook.



http://info.gbiosciences.com/complete-bioassay-handbook

For other related products, visit our website at www.GBiosciences.com or contact us.

Last saved: 8/16/2013 CMH

www.GBiosciences.com