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A Geno Technology, Inc. (USA) brand name

β-Galactosidase Assay (CPRG)

(Cat. # 786-651)

INTRODUCTION

β-Galactosidase Assay (CPRG) provides an easy, rapid, and highly sensitive method for determining the β-galactosidase activity in the lysates of cells transfected with a β-galactosidase expression construct. The β-Galactosidase Assay (CPRG) assay uses chlorophenol red-β-D-galactopyranoside (CPRG), which is up to 10 times more sensitive than the classic *o*-nitrophenyl-β-D-galactopyranoside (ONPG) β-Galactosidase assays. This increased sensitivity makes it simpler to measure β-galactosidase activity in cells that are difficult to transfect or have low β-galactosidase activity expression.

β-galactosidase catalyzes the hydrolysis of the galactoside analog chlorophenol red-β-D-galactopyranoside (CPRG). Cell lysates are incubated with the supplied reagents and the β-Galactosidase converts the yellow-orange CPRG substrate into the red chromophore chlorophenol red, yielding a dark red solution. The β-galactosidase gene functions well as a reporter gene because the protein product is extremely stable, resistant to proteolytic degradation in cellular lysates, and easily assayed. The levels of activity β-galactosidase expression can be quickly measured by this method.

β-Galactosidase activity is measured using a spectrophotometer or a microplate reader to determine the amount of substrate converted at 570–595 nm. Although the CPRG β-Galactosidase Assay Kit provides all the required reagents for 500 micro-assays in a 96-well plate, it is easily adapted for different assay size.

KIT COMPONENTS (Cat. # 786-651)

Description	Size
Mammalian Cell *PE LB*™	100ml
CPRG Assay Reaction Buffer	100ml
CPRG Assay Stop Solution	50ml
CPRG substrate	5 x 16.5mg

STORAGE

The CPRG Substrate 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 microtiter plates
- Protein concentration assay kit, we recommend or CB-X™ single tube protein assay (Cat. # 786-12X) or our NI™ (Non-Interfering™) Protein Assay (Cat. # 786-005)
- Additional Mammalian Cell PE LB™ (Cat. # 786-180) may be purchased if required

PROTOCOLS

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.

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.

β-Galactosidase Assay

CAUTION: *Wear gloves throughout as the CPRG reagent will stain skin.*

1. If frozen, allow the cell lysates to thaw at room temperature.
2. Add 20µl clarified cell lysates to the wells of a 96-well plate. We recommend performing the assay in triplicate. Add 20µl Mammalian *PE LB*™ to three wells that will act as your blank.
   NOTE: *For cuvette size assay, use 100µl lysates.*
3. Prepare 25X CPRG Substrate by adding 550µl of CPRG Assay Reaction Buffer into a vial of CPRG Substrate. To completely dissolve the substrate, mix the vial by vortexing for 20 seconds.
4. For 100 wells, dilute the 25X CPRG Substrate to 1X CPRG Substrate by adding 550µl 25X CPRG Substrate to 13.2ml CPRG Assay Reaction Buffer. You need 130µl 1X CPRG Substrate per well.
   NOTE: *The 25X CPRG Substrate can be stored at -20°C for 1 month. Discard unused 1×CPRG substrate.*
5. Add 130µl of 1X CPRG Substrate to each well and record the time of CPRG Substrate addition. Cover the plate with aluminum foil.
   NOTE: *For cuvette size assay, use 900µl 1X CPRG Substrate.*
6. Incubate the reactions for 30 minutes or longer (up to 72 hours) in a 37°C incubator until the sample turns dark red. The incubation period will vary depending on cell type, transfection rates, etc.
7. Stop the reactions by adding 80µl of stop solution to each well. Read the plates within two hours of adding the stop solution
   NOTE: *For cuvette size assay, use 500µl Stop Solution..*
8. Read in a plate reader at a wavelength of 570–595 nm. Use the blank to zero the plate reader.
   NOTE: *For cuvette size assay, use a suitable spectrophotometer..*

Calculation of Specific activity β-Galactosidase (U/mg)

NOTE: *To calculate this, you will need to determine the protein concentration of the initial cell lysates. We recommend or CB-X™ single tube protein assay (Cat. # 786-12X) or our NI™ (Non-Interfering™) Protein Assay (Cat. # 786-005).*

1. Calculate the average absorbances for samples performed in triplicate or duplicate.
2. Subtract the mock-transfected cell lysate absorbances from the readings to control for endogenous β-galactosidase.
3. Calculate the Specific activity β galactosidase (U/mg) with the following equations:

   [Chlorophenol red formed] (nmol/ml)= Absorbance x 55

   Amount of Chlorophenol red formed (nmol)= [Chlorophenol red formed] (nmol/ml) x total assay Volume (0.23ml or 1.5ml)

   Activity of β-galactosidase (nmol/min or U)= Amount of Chlorophenol red formed (nmol)/ Time of 37°C incubation (min)

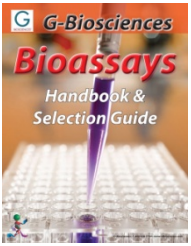
   Specific activity β galactosidase (U/mg)= (Activity of β-galactosidase (U/ protein conc of lysate (mg/μl)))/ volume of lysate in assay (μl)

TROUBLESHOOTING

Observation	Suggestion
No color development	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.
Color development 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.
Sample turns dark red immediately after adding the 1X CPRG substrate	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/

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