CELL APPLICATIONS, INC.

β-Galactosidase Assay Kit: Red (CPRG)

Cat. No. GA10-100K 500 micro assays/96-well plate

*Lac*Z is a commonly used reporter gene in transfection experiments because the gene product, β-galactosidase, is very stable, resistant to proteolytic degradation, and easily assayed. The levels of active β-galactosidase expression can be quickly measured by its catalytic hydrolysis of Chlorophenol red-β-D-galactopyranoside (CPRG) substrate to a dark red product. The assay kit provides all the required reagents, and offers a rapid, simple and sensitive method to quantify the enzyme expression in β-gal-transfected cells. The high sensitivity improves the measurement of β-gal activity when the reporter gene expression is low.

Component	Quantity	Storage (C)
5x Lysis Buffer	55 ml	4
Standard Dilution Buffer	55 ml	4
10x CPRG Substrate Stock (Chlorophenol red-β-D-galactopyranosidase)	5x 1 ml	-20
Substrate Buffer	55 ml	4
Stop Buffer	55 ml	4
β-gal enzyme standard, 40 units	100 µl	-20

EXAMPLE PROTOCOLS

Dilute 5X Lysis buffer to 1X with distilled deionized water before use. Unused 1X Lysis Buffer may be stored at 4°C for future use.

Harvesting adherent cells:

1. Aspirate the growth medium 24-72 hours after transfection from the culture dish including mock transfected control cells (non-transfected cells). Cells can be optionally washed 1 time with 1x PBS.

2. Add 1x Lysis Buffer to the culture dish. Solution volumes recommended for various culture dishes are listed in the following table.

Table 1.

Tissue Culture Dish	Volume 1x Lysis Buffer (µl/well)
96-well	50
24-well	250
12-well	500
6-well	1000
60 mm	2500
100 mm	5000

3. Incubate the dish 10-15 minutes at room temperature by swirling it slowly several times to ensure complete lysis. The culture dishes can be observed under a microscope to confirm that the cells are lysed completely. Lysate is ready for colorimetric analysis. Plates or dishes can be optionally centrifuged for 2-3 minutes to pellet the insoluble material.

NOTE: A quick freeze-thaw cycle of the dish (freeze 1-2 hours at –20°C or -70°C, and thaw at room temperature) can also be done to obtain a good lysis. Proceed to the colorimetric assay or freeze the plate at –70°C until ready.

Harvesting suspension cells:

1. Aspirate the supernatant 24-72 hours post-transfection after centrifugation at 250 x g for 5 minutes. Cells pellet can be optionally washed 1 time with 1x PBS.

2. Resuspend the cell pellet in 1x Lysis Buffer. The amount of Lysis Buffer depends on the size of the culture dishes used for transfection (i.e., cell pellet size) and we recommend using between 50 to 2000 µl.

3. Incubate the cell lysate 10-15 minutes at room temperature by gently swirling the tubes or dishes several times to ensure complete lysis. Proceed to the colorimetric assay or freeze the plate at –70°C till ready. Plates or dishes can be optionally centrifuged for 2-3 minutes to pellet the insoluble material.

NOTE: A quick freeze/thaw cycle (freeze 1-2 hours at –20°C or –70°C and thaw at room temperature) can also be done to obtain a good lysis.

Dilute 10X CPRG stock to 1X with Substrate Buffer just before performing the colorimetric assay. Unused 1X CPRG may be stored at –20C for future use. We recommend using 1X CPRG solution only 2 times after a freeze/thaw cycle.

CAUTION: Wear Gloves when manipulating the CPRG since it will stain exposed skin.

96-well microtiter plate assay*

1. Thaw the dish, tube, or plate of lysed cells at room temperature. If the transfection is performed with a 96-well plate, perform the assay directly on the plate.

2. Add 50 µl of Standard Dilution Buffer to the wells of a 96-well plate (flat bottom), except control wells, which are set aside for establishing a standard curve.

3. Prepare a serial dilution of β-galactosidase (*E.coli*) standards with Standard Dilution Buffer separately (See Table 2). A 50 µl aliquot of each point on the standard curve is transferred to the control wells of the plate - the highest recommended amount of β-galactosidase is 100 milliunits (100,000~200,000 pg). 2x serial dilution of standard curve consisting of 8 points is recommended. An example of a serial dilution protocol is shown

in Table 2. Standards are made in serial dilutions: 5 µl of the β-gal Enzyme Standard Stock Solution is used to make the 100 mu β-gal Enzyme Standard, 200 µl of the 100 mu standard is used to make the 50 µl standard, etc.

Table 2.

β-gal Standard (milliunits)	Standard Dilution Buffer Volume (µl)	Volume of serially diluted β-gal Enzyme standards
100 mu	995	5 µl β-gal enzyme standard stock
50	200	200 µl of 100 mu β-gal standard
25	200	200 µl of 50 mu β-gal standard
12.5	200	200 µl of 25 mu β-gal standard
6.25	200	200 µl of 12.5 mu β-gal standard
3.125	200	200 µl of 6.25 mu β-gal standard
1.562	200	200 µl of 3.125 mu β-gal standard
0.78	200	200 µl of 1.562 mu β-gal standard

NOTE:
• Adjust the standard curve to suit the specific experimental conditions, such as cell type, transfection reagent, or plasmid vector.
• The dilutions for the standard curve must be prepared freshly each time the assay is performed.

4. Add 50 µl of each sample/well. Prepare a blank by adding 50 µl of lysis buffer to a well. Also add 50 µl of cell lysate from non-transfected cells (mock-transfected cells) to a well as a control for endogenous β-galactosidase activity.

NOTE: It may be necessary to dilute the cell lysate in 1x Lysis Buffer when transfection efficiency is very high. In contrast, when transfection efficiency is very low, reduce the volume of lysis buffer used to harvest the cells (see description above) or use up to 150 µl of cell extract for the colorimetric assay. If the transfection is performed with a 96-well plate, perform the assay directly on the plate.

5. Add 100 µl of 1x CPRG Substrate Solution to each well. Incubate the plate at room temperature until the dark red color develops (depending on cell type, this may require an incubation time from 10 minutes to up to 4 hours).

6. Read the absorbance at 570-595 nm with a microtiter spectrophotometer. Stop solution is not required for this format, since all wells are read simultaneously without a time gap. Be sure that there are no bubbles present in the wells while reading. Bubbles will interfere with the absorbance reading and can be removed with a fine gauge needle, tips or very weak gas flow.

7. Quantify β-galactosidase expression based on a linear standard curve.

* *Felgner, J.H. et al. Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. J. Biol. Chem. 269, 2550-2561 (1994).*

Macro assay

1. Prepare a blank by adding 100 µl of lysis buffer to a well. Thaw the cell lysates and transfer 100 µl to fresh tubes. Add 100 µl of cell lysate from non-transfected cells (mock-transfected cells) to a tube to control endogenous β-galactosidase activity. If a 96-well plate is used, follow the Microtiter Plate protocol described above.

NOTE: It may be necessary to dilute the cell lysate in 1x Lysis Buffer when transfection efficiency is very high. In contrast, when transfection efficiency is very low, reduce the volume of lysis buffer used to harvest the cells (see description above) or use up to 150 µl of cell extract for the colorimetric assay.

2. Add 50 µl of Standard Dilution Buffer to each tube.

3. Prepare a serial dilution of β-galactosidase (*E.coli*) standards with Standard Dilution Buffer separately. Transfer 50 µl of each standard to a fresh tube containing 100 µl cell lysate from a mock transfection. The highest recommended amount of beta-galactosidase is 200,000 pg. (100 miliunits). Adjust the standard curve to suit the specific experimental conditions, such as cell type, transfection reagent, or plasmid vector. 2X serial dilution of standard curve consisting of 8 points is recommended. A dilution protocol example is shown in the section of 96-well plate assay.

4. Add 300 µl of 1x CPRG Substrate Solution to each tube. Incubate the tubes at room temperature till the red color develops (from approximately 10 minutes to 4 hours depending on the cell type). Add 500 µl of Stop Solution to stop the reaction. Final volume is 950 µl.

5. Read the absorbance at 570-595 nm with a spectrophotometer.

6. Quantify β-galactosidase expression based on a linear standard curve.