CELL APPLICATIONS, INC.

β-Galactosidase Assay Kit: Yellow (ONPG)

Cat. No. GA10-200K 500 micro assays/96-well plate

LacZ is a commonly used reporter gene in transfection experiments because the gene product, β-galactosidase, is very stable and resistant to proteolytic degradation and easily assayed. The levels of active β-galactosidase expression can be easily measured by its catalytic hydrolysis activity of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) substrate to a bright yellow product. The assay kit provides all the required reagents, and offers a rapid, simple and sensitive method to quantify the enzyme expression in *lacZ*-transfected cells. Sufficient reagents are provided to perform 500 microassays in a 96-well plate.

Component	Quantity	Storage (°C)
5x Lysis Buffer	55 ml	4
Standard Dilution Buffer	55 ml	4
ONPG Substrate Stock (*o*-nitrophenyl-β-D-galactopyranosidase)	55 ml	4
Stop Buffer	55 ml	4
β-gal enzyme standard, 40 units	100 µl	-20

PROTOCOLS

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

Harvesting adherent cells:

1. Aspirate the growth medium 24-72 hours after transfection from the culture dish including mock transfected 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.

Tissue Culture Dish	Volume 1x Lysis Buffer (ul/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.

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

Optional: Before proceeding to the colorimetric assay, the plate or dish can be centrifuged for 2-3 minutes to pellet the insoluble material. Then, the supernatant is ready to be assayed.

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 dishes several times to ensure complete lysis. Proceed to the colorimetric assay or freeze the plate at –70°C till ready.

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.

Optional: Before proceeding to the colorimetric assay, the tube containing the cell lysate can be centrifuged for 2-3 minutes to pellet the insoluble material. Then, the supernatant is ready to be assayed.

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 creating a standard curve.

3. Prepare a serial dilution of β-galactosidase (*E.coli*) standards with Standard Dilution Buffer separately. 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 200 milliunits (200,000~400,000 pg). 2x serial dilution of standard curve consisting of 8 points is recommended. A dilution protocol example is shown in the following table.

β-gal Standard (milliunits)	Standard Dilution Buffer Volume (µl)	β-gal Standard Volume
200	990	10 µl β-gal standard stock
100	200	200 µl of 200 mu β-gal standard
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

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.

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.

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

5. Add 100 µl of ONPG Substrate Solution to each well. Incubate the plate at room temperature until the yellow color develops (from approximately 10 minutes to 4 hours depending on the cell type).

6. Read the absorbance at 405-420 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, tip 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. Thaw the cell lysate and transfer 100 µl to a fresh tube, or 50 µl to a 96-well plate. If a 96-well plate is used, follow the 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.

Prepare a blank by adding 100 ul of lysis buffer to a tube. Add also 100ul of cell lysate from non-transfected cells (mock-transfected cells) to a tube to control endogenous β-galactosidase activity.

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 200,000pg. (100 milliunits). 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 ONPG Substrate Solution to each tube. Incubate the tubes at room temperature till the yellow 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 405-420 nm with a spectrophotometer.

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