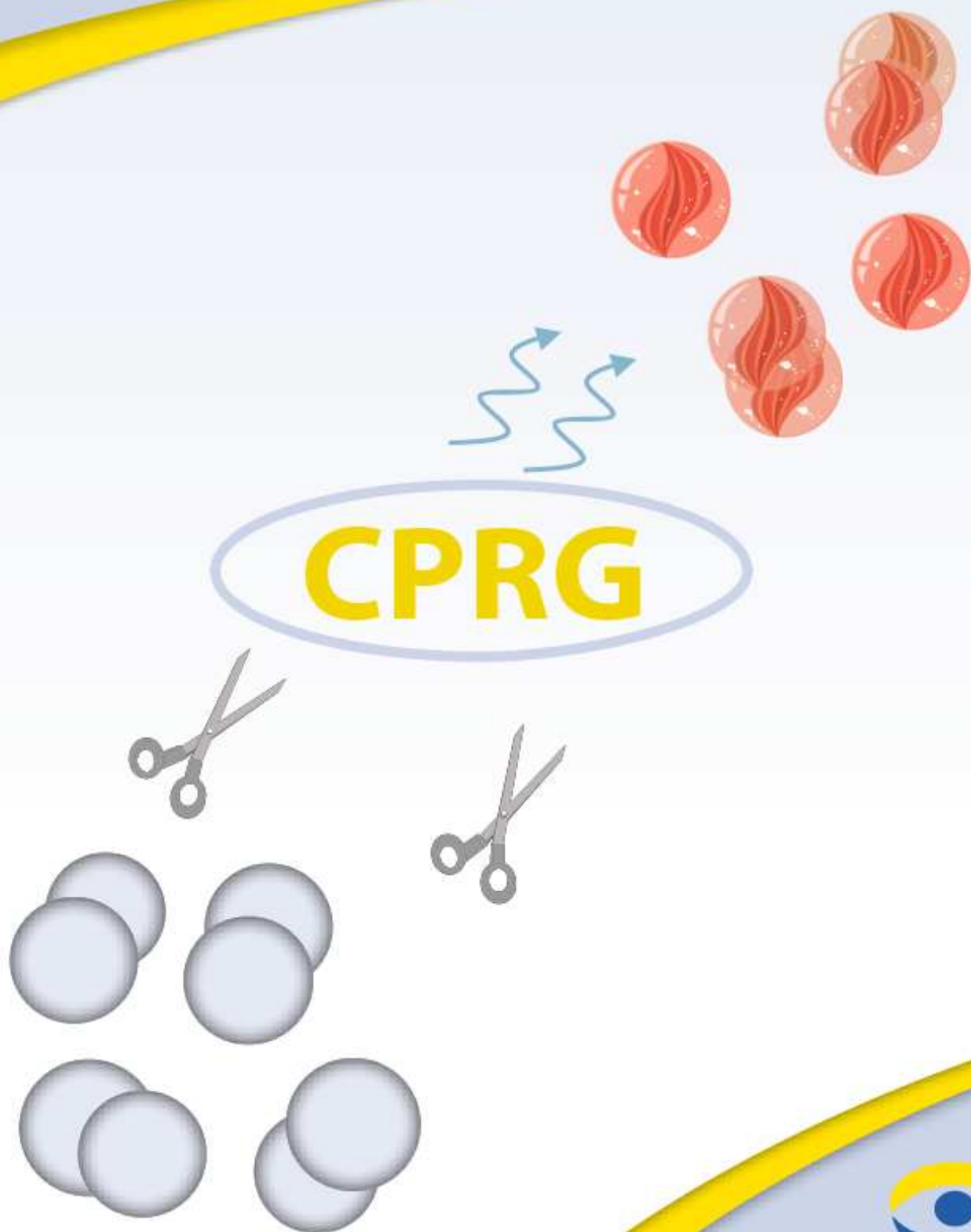


CPRG β -Galactosidase Assay Kit

INSTRUCTION MANUAL



β -Galactosidase

CPRG β -Galactosidase Assay Kit

Instruction Manual

Ready-to-use assay system for quantitatively measuring β -galactosidase expression levels in transfected cells using a highly sensitive substrate, CPRG.

Catalog Number: GC10002

You can order this product by contacting us. For all other additional information, do not hesitate to contact our dedicated technical support (tech@ozbiosciences.com).

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1. Technology

1.1. Description

Congratulations on your purchase of the CPRG β -galactosidase assay kit!

LacZ is one of the most frequently used reporter gene in transfection experiments because of the gene product specific properties. Indeed, the LacZ encoded protein, β -galactosidase, is very stable, resistant to proteolytic degradation and easily tested. 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. All the necessary reagents provided in this assay kit offer a rapid, simple and sensitive method to quantify the enzyme expression level in cells. The high sensitivity improves the measurement of β -galactosidase activity when the reporter gene expression is low.

This CPRG β -galactosidase assay kit is:

- Simple and Rapid
- Ready-to-use
- Economical

1.2. Kit Contents

The kit is provided with sufficient reagents to perform 500 micro assays in 96-well plate.

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

Stability and Storage

Storage Upon receipt and for long-term use, store all reagent tubes at the indicated storage conditions (see table above). Kit's components are stable for at least 1 year at the recommended storage temperature.

Shipping condition The CPRG β -galactosidase assay kit is shipped with gel pack (4°C)

2. Applications and Protocols

2.1. Usage

1. Transfect cells with a plasmid expressing Lac Z gene
2. Lyse the cells using the lysis buffer
3. Transfer the lysate to a fresh tube or a micro titer plate. Dilute the lysate if needed
4. Prepare a β -galactosidase standard curve with standard dilution buffer
5. Add the substrate and monitor the color development at 570-595 nm
6. Calculate the expression levels based on a standard curve

2.2. General Considerations

- Before use, dilute the 5X Lysis buffer to 1X with distilled water. The surplus of unused 1X Lysis Buffer may be stored at +4°C for future use.
- Just before performing the colorimetric assay, dilute the 10X CPRG stock solution to 1X with the Substrate Buffer. The surplus of unused 1X CPRG may be stored at –20°C for future use. We recommend using 1X CPRG solution only 2 times after a freeze/thaw cycle.

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

2.3. General Protocol

- **Harvesting adherent cells:**

1. Aspirate the growth medium 24-72 hours after transfection from the culture dish including the control cells (non-transfected). Cells can be optionally washed once with 1X PBS.
2. Add 1X Lysis Buffer to the culture dish. Solution volumes recommended for various plates are:

Type of culture dish	Volume of 1X Lysis Buffer (μ L / well)
96-well plate	50
24-well plate	250
12-well plate	500
6-well plate	1000
60 mm dish	2500
100 mm dish	5000

3. Incubate the dish 10-15 min. at room temperature by swirling it slowly several times to ensure complete lysis. The dishes can be observed under a microscope to confirm the complete lysis.

NOTE: A fast 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 achieve a good lysis. Proceed to the colorimetric assay or freeze the plate at -70°C until ready.

- OPTIONAL:** The dish can be centrifuged 2 min. to pellet the insoluble material before proceeding to the colorimetric assay. The supernatant is ready to be tested.

- Harvesting suspension cells:**

- 24-72 hours post-transfection, centrifuge the cells at $250 \times g$ for 5 minutes. Then, aspirate the supernatant. Cells pellet can be optionally washed once with 1X PBS.
- 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 .
- Incubate the cell lysate 10-15 min. 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 until 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:** The dish can be centrifuged 2 min. to pellet the insoluble material before proceeding to the colorimetric assay. Then, the supernatant is ready to be assayed.

2.4. 96-well micro titer plate

- If needed 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.
- Add 50 μL of Standard Dilution Buffer to the wells of a 96-well plate (flat bottom) except the control wells, which are save for the standard curve.
- In different tubes or well, prepare a serial dilution of β -galactosidase (E. Coli) standards with Standard Dilution Buffer. Then, transferred 50 μL aliquot of each point on the standard curve 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. A dilution protocol example is shown in the following table.

β -Gal Standard (milliunits)	Standard Dilution Buffer Volume	β -Gal Standard Volume
100	995 μL	5 μL of β -gal standard stock
50	200 μL	200 μL of 100 mu β -gal standard
25	200 μL	200 μL of 50 mu β -gal standard
12.5	200 μL	200 μL of 25 mu β -gal standard
6.25	200 μL	200 μL of 12.5 mu β -gal standard
3.125	200 μL	200 μL of 6.25 mu β -gal standard
1.562	200 μL	200 μL of 3.125 mu β -gal standard
0.78	200 μL	200 μL of 1.562 mu β -gal standard

NOTE 1: Adjust the standard curve to suit the specific experimental conditions, such as cell type, cell number, transfection reagent, size of the culture dish or plasmid vector.

NOTE 2: 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 required to dilute the lysate in 1X Lysis Buffer when transfection efficiency is very high. In contrast, when transfection efficiency is low, reduce the volume of lysis buffer used to harvest the cells (see above) or use up to 150 μL of cell lysate for the colorimetric assay. If the transfection is performed with a 96-well plate, perform the assay directly on the plate.

To control endogenous β -galactosidase activity, prepare controls (blank) by adding 50 μL of lysis buffer to a well and 50 μL of cell lysate from non-transfected cells to another well.

5. Add 100 μL of 1X CPRG Substrate Solution to each well. Incubate the plate at room temperature until the dark red color develops (from approximately 10 min. to 4 h depending on the cell type).
6. Read the absorbance at 570-595 nm with a micro titer spectrophotometer. Stop solution is not required for this format, since all wells are read simultaneously. Avoid the presence of bubbles 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).

2.5. Macro assay

1. Thaw the cell lysate (if needed) 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 μL of lysis buffer to a tube. Add also 100 μL 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 β -galactosidase is 200,000 pg. (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 CPRG Substrate Solution to each tube. Incubate the tubes at room temperature until 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.

3. Related Products

Description	Reference
Tee-Technology (lipid-based reagents)	
DreamFect Transfection reagent 1mL	DF41000
DreamFect Gold Transfection reagent 1mL	DG81000
Lullaby siRNA Transfection Reagent 1mL	LL71000
EcoTransfect Transfection Reagent 1mL	ET11000
VeroFect Transfection Reagent 1mL	VF61000
FlyFectin Transfection Reagent 1mL	FF51000
Magnetofection Technology	
Mega Magnetic Plate	MF14000
Super Magnetic Plate	MF10000
Magnetic Plate 96-magnets	MF10096
PolyMag 1mL (<i>for all nucleic acids</i>)	PN31000
PolyMag Neo 1mL (<i>for all nucleic acids</i>)	PG61000
CombiMag 1mL (<i>to boost transfection reagent</i>)	CM21000
SilenceMag 1mL (<i>for siRNA application</i>)	SM11000
ViroMag 1mL (<i>for viral application</i>)	VM41000
ViroMag R/L 1mL (<i>for lenti- & retro-viruses</i>)	RL41000
AdenoMag 1ml (<i>for adeno viruses</i>)	AM71000
NeuroMag 1mL (<i>for transfection of neurons</i>)	NM51000
SelfMag Amino Kit	SA10000
SelfMag Carboxy Kit	SC20000
FluoMag-P 100 μ L	FP10100
FluoMag-C 100 μ L	FC10100
FluoMag-S 100 μ L	FS10100
FluoMag-V 100 μ L	FV10100
DeliverIN Technology	
Ab-DeliverIN 1 mL	AI21000
Pro-DeliverIN 1 mL	PI11000
Gene & Protein Tools	
Bradford – Protein Assay Kit	BA00100
GeneBlaster™ Ruby	GB20011
GeneBlaster™ Sapphire	GB20012
GeneBlaster™ Topaz	GB20013
GeneBlaster™ Emerald	GB20014
shRNA GFP (pure)	SH10001
shRNA Luciferase (pure)	SH10002
X-Gal Staining Kit	GX10003
β -Galactosidase (ONPG) assay kits	GO10001
β -Galactosidase (CPRG) assay kits	GC10002

Do not hesitate to contact us for all complementary information and remember to visit our website in order to stay informed on our latest breakthrough technologies and updated on our complete product list.
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