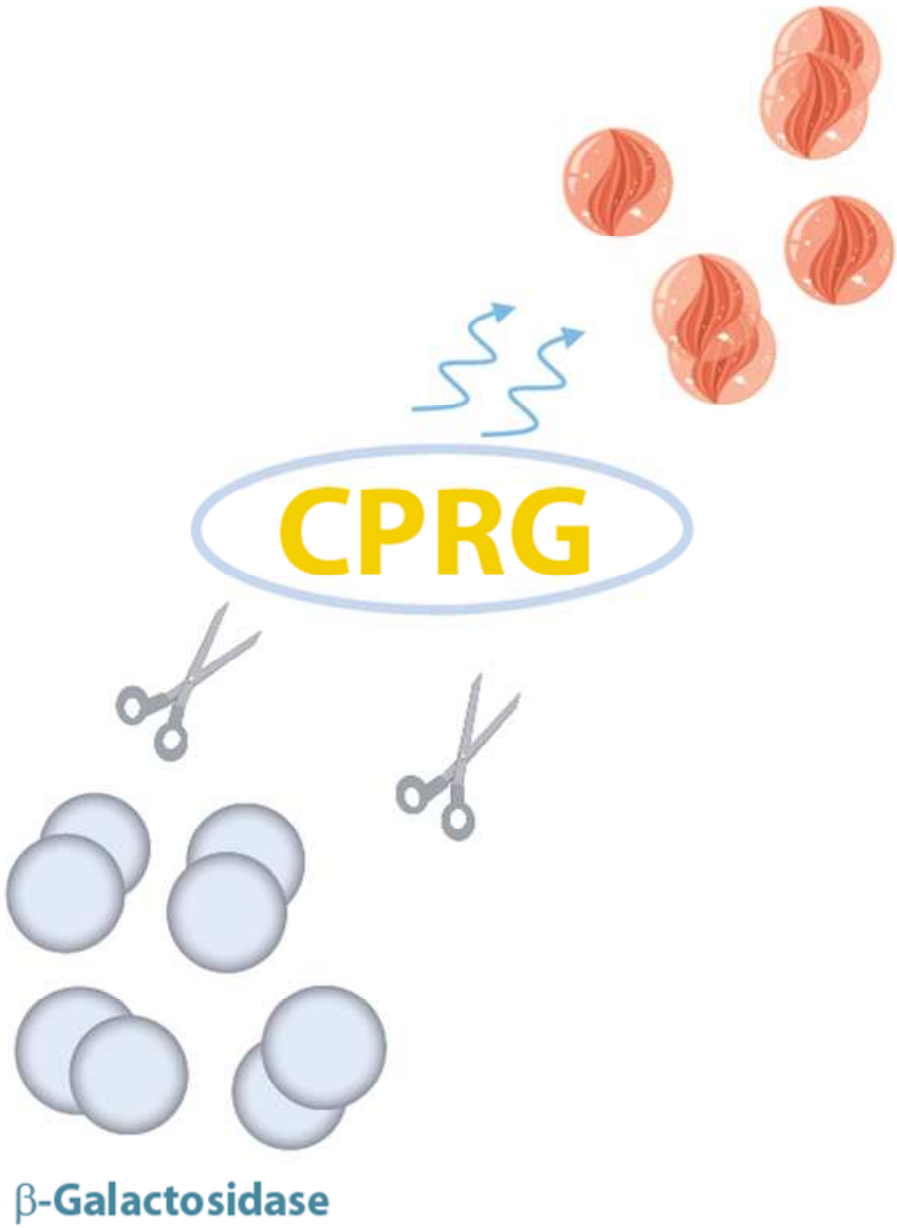
# CPRG β-Galactosidase Assay Kit

# INSTRUCTION MANUAL

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# 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 µL | -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 (µL / 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.

4. **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:**

1. 24-72 hours post-transfection, centrifuge the cells at 250 x g for 5 minutes. Then, aspirate the supernatant. Cells pellet can be optionally washed once 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 L.
3. 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.
4. **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 assay*

1. 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.

2. 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.

3. 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 L 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 L 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 L of lysis buffer to a well and 50 L 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 ß-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 |
|---|
| **MAGNETOFECTION TECHNOLOGY** |
| Super Magnetic Plate *(standard size for all cell culture support)*<br>Mega Magnetic plate *(mega size to hold 4 culture dishes at one time)* |
| **Transfection reagents**: |
| PolyMag Neo (*for all nucleic acids*) |
| Magnetofectamine™ (*for all nucleic acids*) |
| NeuroMag *(dedicated for neurons)* |
| SilenceMag *(for siRNA application)* |
| **Transfection enhancer:** |
| CombiMag *(to improve any transfection reagent efficiency)* |
| **Viral Transduction enhancers:** |
| ViroMag (*to optimize viral transduction*) |
| ViroMag R/L *(specific for Retrovirus and Lentivirus)* |
| AdenoMag *(for Adenoviruses)* |
| **LIPOFECTION TECHNOLOGY (LIPID-BASED)** |
| Lullaby (*siRNA transfection reagent)* |
| DreamFect Gold *(Transfection reagent for all types of nucleic acids)* |
| VeroFect *(for Vero cells)* |
| FlyFectin *(for Insect cells)* |
| **i-MICST TECHNOLOGY** |
| Viro-MICST (*to transduce directly on magnetic cell purification columns)* |
| **3D TRANSFECTION TECHNOLOGY** |
| 3Dfect *(for scaffolds culture)* / 3DfectIN *(for hydrogels culture)* |
| **RECOMBINANT PROTEIN PRODUCTION** |
| HYPE-5 Transfection Kit (*for **H**igh **Y**ield **P**rotein **E**xpression)* |
| **PROTEIN DELIVERY SYSTEMS** |
| Ab-DeliverIN *(delivery reagent for antibodies)*<br>Pro-DeliverIN *(delivery reagent for protein in vivo and in vitro)* |
| **PLASMIDS PVECTOZ** |
| pVectOZ-LacZ / pVectOZ-SEAP / pVectOZ-GFP / pVectOZ-Luciferase |
| **ASSAY KITS** |
| Bradford – Protein Assay Kit<br>MTT cell proliferation kit<br>β-Galactosidase assay kits (CPRG/ONPG) |
| **BIOCHEMICALS** |
| D-Luciferin, $K^+$ and $Na^+$ 1g<br>X-Gal powder 1g / G-418, Sulfate 1g |

Do not hesitate to contact us for all complementary information and remember to visit our website in order to stay inform on our last breakthrough technologies and updated on our complete product list.
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# Purchaser Notification

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