# High Sensitivity β-Galactosidase Assay Kit

## Instruction Manual

**Catalog #200710**

Revision B

**Research Use Only. Not for Use in Diagnostic Procedures.**

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# High Sensitivity β-Galactosidase Assay Kit

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# High Sensitivity β-Galactosidase Assay Kit

## MATERIALS PROVIDED

| Material provided[a] | Quantity | Storage condition[b] |
|---|---|---|
| CPRG substrate (chlorophenol red-β-D-galactopyranoside) | 4 × 30 mg | –20°C |
| Buffer A | 100 ml | Room temperature |
| Stop solution | 50 ml | Room temperature |
| Lysis buffer | 50 ml | Room temperature |

[a] Sufficient reagents are provided to perform 100 standard assays in 60-mm dishes or 600 microassays in 96-well microtiter dishes.

[b] The solutions are stable for 6 months when stored at the indicated temperatures.

## STORAGE CONDITIONS

CPRG Substrate: **–20°C**
Stop Solution: **Room temperature**
Lysis Buffer: **Room temperature**
Buffer A: **Room temperature**

***Caution*** *Wear gloves. The CPRG substrate will stain exposed skin.*

## ADDITIONAL MATERIALS REQUIRED

Phosphate-buffered saline (PBS)[§]
Disposable cuvettes or 96-well microtiter dishes
Protein concentration assay kit

[§] See *Preparation of Media and Reagents*.

Revision B

# INTRODUCTION

The enzyme β-galactosidase catalyzes the hydrolysis of β-galactosides, including lactose and the galactoside analog chlorophenol red-β-D-galactopyranoside (CPRG). 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 High Sensitivity β-Galactosidase Assay Kit provides an easy, rapid, and sensitive method for determining the β-galactosidase activity in the lysates of cells transfected with a β-galactosidase expression construct.[1–4] Cell lysates are simply incubated with a reaction buffer and CPRG substrate. β-Galactosidase converts the yellow-orange CPRG substrate into galactose and the chromophore chlorophenol red, yielding a dark red solution. In contrast to luciferase assays, no expensive equipment is needed to assay β-galactosidase activity. β-Galactosidase activity is quantitated using a spectrophotometer or a microplate reader to determine the amount of substrate converted at 570–595 nm (570 nm is the absorbance maximum). The High Sensitivity β-Galactosidase Assay Kit is up to 10 times more sensitive than β-galactosidase assay methods that use *o*-nitrophenyl-β-D-galactopyranoside (ONPG) as a substrate under the same conditions.[2] The high sensitivity is critical for the measurement of β-galactosidase in cells that are difficult to transfect or have low β-galactosidase expression.

# CELL-HARVESTING PROTOCOLS

## Harvesting Adherent Cells

***Notes*** *The following procedure is optimized for 60-mm tissue culture dishes. See* Table I *for solution volumes for dishes of other dimensions.*

*If the cells are loosely adhered to the dish, aspirate the media from the dish and begin with step 2.*

1. Wash each 60-mm tissue culture dish of transfected cells with 5 ml of 1× PBS. Aspirate the PBS from the dishes. Prepare a control sample for endogenous β-galactosidase activity by washing a 60-mm dish of mock-transfected cells with 5 ml of 1× PBS and aspirating the PBS from the dish.

2. Add 500 µl of lysis buffer to each 60-mm dish.

3. Freeze the dishes at –20°C for 30 minutes.

4. Thaw the dishes at room temperature.

5. Evaluate the tissue culture dishes under a microscope to confirm that the cells are lysed completely. If cell lysis is incomplete, repeat the freeze–thaw cycle or add Triton® X-100 to the lysis buffer to a concentration of 1%.

6. Transfer the cell lysates to 1.5-ml microcentrifuge tubes.

7. Spin the cell lysate in a centrifuge at 12,000 × *g* for 5 minutes at 4°C to pellet the cell debris.

8. Transfer the supernatant from each tube to a fresh microcentrifuge tube and store at –20°C or proceed with the *Micro* or *Macro β-Galactosidase Assay.*

**TABLE I**

| Format of tissue culture dish | Volume of 1× PBS wash (ml/dish or well) | Volume of lysis buffer (µl/dish or well) |
|---|---|---|
| 35-mm dish | 2.5 | 250 |
| 60-mm dish | 5 | 500 |
| 100-mm dish | 10 | 1000 |
| 150-mm dish | 25 | 2500 |
| 6-well plate | 2.5 | 250 |
| 12-well plate | 1 | 100 |
| 24-well plate | 0.5 | 50 |
| 48-well plate | 0.25 | 20 |
| 96-well plate | 0.1 | 10 |

### Harvesting Suspended Cells

1. Transfer the transfected cells to 15-ml centrifuge tubes. Prepare a control sample for endogenous β-galactosidase activity by adding mock-transfected cells to a 15-ml centrifuge tube.

2. Spin the tubes at 200 × *g* for 5 minutes to pellet the cells. After centrifugation, aspirate the supernatant from the tubes.

3. Gently wash the cell pellets by resuspending the cells in 5 ml of 1× PBS.

4. Spin the tubes at 200 × *g* for 5 minutes to pellet the cells. After centrifugation, aspirate the supernatant from the tubes.

5. Add 500 µl of lysis buffer to each centrifuge tube and vortex for 5 seconds.

6. Freeze the tubes of cell lysate at –20°C for 30 minutes.

7. Thaw the tubes of cell lysate at room temperature.

8. Transfer the cell lysate in each tube to a 1.5-ml microcentrifuge tube.

9. Spin the tubes in a centrifuge at 12,000 × g for 5 minutes at 4°C to pellet the cell debris.

10. Transfer the supernatant from each tube to a fresh microcentrifuge tube and store at –20°C or proceed with the *Micro* or *Macro β-Galactosidase Assay.*

## β-GALACTOSIDASE ASSAY PROTOCOLS

### Micro β-Galactosidase Assay

***Caution*** *Wear gloves. The CPRG substrate will stain exposed skin.*

***Notes*** *We recommend assaying samples in duplicate or triplicate.*

*To minimize bubbles in the wells, which can affect the optical density readings, use the reverse-pipetting technique to load the samples into the wells (see* Appendix: Reverse-Pipetting Technique*).*

1. Pipet 20 µl of cell lysate into the wells of a 96-well microtiter dish. Prepare a blank (for zeroing the microtiter dish reader) by adding 20 µl of lysis buffer to a well. Prepare the control for endogenous β-galactosidase activity by adding 20 µl of lysate from the mock-transfected cells to a well.

2. Prepare 25× CPRG substrate by adding 1 ml of buffer A to a vial of CPRG substrate. Mix the two components by inversion. Dilute the 25× CPRG substrate to 1× CPRG substrate by adding 1 part of 25× CPRG substrate to 24 parts of buffer A.

   **Note** *The 25× CPRG substrate can be stored at –20°C for 1 month. Discard unused 1× CPRG substrate.*

3. Add 130 µl of 1× CPRG substrate to each well for a final volume of 150 µl. Record the time of CPRG substrate addition. Cover the dish with a microplate lid.

4. 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.

5. Terminate the reactions by adding 80 µl of stop solution to each well. Record the incubation period, which is the time expired between the addition of CPRG substrate and the addition of the stop solution.

   **Note** *Because the CPRG substrate begins to oxidize after stop solution is added, perform step 6 within 2 hours.*

6. Scan the microtiter dish in a microtiter dish reader at a wavelength of 570–595 nm. Use the blank to zero the microtiter dish reader. Alternatively, measure the optical density ($OD_{570–595}$) of the blank and subtract it from the $OD_{570–595}$ value of each well of cell lysate.

7. Determine the protein concentration and the specific activity of the sample (see *Calculating the Specific Activity of β-Galactosidase)*.

## Macro β-Galactosidase Assay

**Caution** *Wear gloves. The CPRG substrate will stain exposed skin.*

1. Pipet 100 µl of cell lysate into a 1.5-ml microcentrifuge tube. Prepare a blank (for zeroing the spectrophotometer) by adding 100 µl of lysis buffer to a 1.5-ml microcentrifuge tube. Prepare the control for endogenous β-galactosidase activity by adding 100 µl of lysate from the mock-transfected cells to a 1.5-ml microcentrifuge tube.

2. Prepare 25× CPRG substrate by adding 1 ml of buffer A to a vial of CPRG substrate. Mix the two components by inversion. Dilute the 25× CPRG substrate to 1× CPRG substrate by adding 1 part of 25× CPRG substrate to 24 parts of buffer A.

   **Note** *The 25× CPRG substrate can be stored at –20°C for 1 month. Discard unused 1× CPRG substrate.*

3. Add 900 µl of 1× CPRG substrate to each tube and vortex for 5 seconds. Record the time of CPRG substrate addition.

4. Incubate the reactions for 30 minutes or longer (up to 72 hours) in a 37°C water bath until the sample turns dark red. The incubation period will vary.

5. Stop the reactions by adding 500 µl of stop solution. Record the incubation period, which is the time expired between the addition of CPRG substrate and the addition of the stop solution.

   **Note** *Because the CPRG substrate begins to oxidize after stop solution is added, perform step 6 within 2 hours.*

6. Measure the optical density of the experimental and control samples at a wavelength of 570–595 nm ($OD_{570–595}$). Use the blank to zero the spectrophotometer. Alternatively, the $OD_{570–595}$ of the blank can be measured and subtracted from the $OD_{570–595}$ value of each tube of cell lysate.

7. Determine the protein concentration and the specific activity of the sample (see *Calculating the Specific Activity of β-Galactosidase)*.

## CALCULATING THE SPECIFIC ACTIVITY OF β-GALACTOSIDASE

The specific activity of β-galactosidase is expressed as nmol of chlorophenol red formed/minute/mg of total protein, which equals U of β-galactosidase activity/mg of total protein. Because the specific activity of a preparation depends on the assay conditions, such as the concentration of the substrate, pH, ionic strength, and assay temperature, the conditions are usually reported along with the specific activities.

**Note** *To calculate the specific activity of β-galactosidase, the quantity of total protein in the cell lysate has to be measured using a protein concentration assay kit.*

1. Concentration of chlorophenol red formed (nmol/ml) = optical density × 55

2. Amount of chlorophenol red formed (nmol) = concentration of chlorophenol red formed (nmol/ml) × total assay volume, including lysate, 1× CPRG, and stop solution (ml)

3. Activity of β-galactosidase (nmol/minute or U) = chlorophenol red formed (nmol)/time length of the 37°C incubation (minutes)

4. Specific activity of β-galactosidase (U/mg) = [β-galactosidase activity (U)/protein concentration of the lysate (mg/µl)]/volume of the lysate used in the assay (µl).

## TROUBLESHOOTING

| Observation | Suggestion |
|---|---|
| No color development | Cell lysis is incomplete. Repeat the freeze–thaw cycle or add Triton X-100 to the lysis buffer to a concentration of 1%. |
| | Cells are not efficiently transfected with the reporter plasmid. Optimize the transfection conditions. |
| | Stain cells for β-galactosidase activity in situ to determine transfection efficiency. |
| | Verify that the assay incubation temperature was 37°C. |
| | 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 | Cell lysate contains a high β-galactosidase concentration. Decrease the assay incubation time. |
| | Decrease the β-galactosidase concentration by using less cell lysate in the assay and diluting the cell lysate with lysis buffer before performing the assay. |
| Sample turns dark red immediately after adding the 1× CPRG substrate | Decrease the β-galactosidase concentration by using less cell lysate in the assay and diluting the cell lysate with lysis buffer before performing the assay. |

## APPENDIX: REVERSE-PIPETTING TECHNIQUE

1. Press the pipettor push button all the way down to the second stop.

2. Immerse the tip in the liquid and slowly release the push button to the full up position.

3. To dispense the liquid, press the push button to the **first stop**. Withdraw the tip from the receiving vessel without dispensing the liquid remaining in the tip.

4. To pipet multiple samples with the reverse-pipetting technique, keep the button at the first stop position after dispensing each sample. Aspirate the next sample by releasing the button to the full up position. Continue with step 3.

   ***Note*** *When finished pipetting all the samples, return the liquid remaining in the tip to the original sample container by pushing the button to the second stop position.*

## PREPARATION OF MEDIA AND REAGENTS

| | |
|---|---|
| **Buffer A (pH 7.5)**<br>25 mM MOPS [3-(N-Morpholino) propanesulfonic acid]<br>100 mM NaCl<br>10 mM $MgCl_2$ | **Lysis Buffer**<br>0.25 M Tris (pH 7.4)<br>0.25% (v/v) NP-40<br>2.5 mM EDTA |
| **1× PBS**<br>137 mM NaCl<br>2.6 mM KCl<br>10 mM $Na_2HPO_4$<br>1.8 mM $KH_2PO_4$<br>Adjust the pH to 7.4 with HCl | **Stop Solution**<br>0.5 M $Na_2CO_3$ |

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## ENDNOTES

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