β–Galactosidase Assay Kit

INSTRUCTION MANUAL

Catalog #200383
Revision A

For In Vitro Use Only
200383-12

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β–Galactosidase Assay Kit

CONTENTS

β–Galactosidase Assay Kit

MATERIALS PROVIDED

Material provided[a]	Quantity
ONPG (o-Nitrophenyl-β-D-galactopyranoside) (4 mg/ml)	20 ml
β-Mercaptoethanol (1 M)	4.5 ml
Buffer A	90 ml
Stop solution	50 ml
Lysis buffer	20 ml

[a] Sufficient reagents are provided to perform 100 standard assays or 400 micro assays.

STORAGE CONDITIONS

ONPG: –20°C
β-Mercaptoethanol: Room temperature
Stop Solution: Room temperature
Lysis Buffer: Room temperature
Buffer A: Room temperature

ADDITIONAL MATERIALS REQUIRED

PBS buffer (phosphate-buffered saline)
Disposable cuvettes
Polystyrene tubes (12 × 75 mm)
Protein assay kit

Revision A

INTRODUCTION

β-Galactosidase is an enzyme that catalyzes the hydrolysis of β-galactosides, including lactose and the galactoside analog o-nitrophenyl-β-D-galactopyranoside (ONPG). The β-galactosidase gene functions well as a reporter gene for two major reasons: its protein product is extremely stable and resistant to proteolytic degradation in cellular lysates, and most importantly, the enzyme is easily assayed. The Stratagene β-Galactosidase Assay Kit provides an easy and rapid method for the assay of β-galactosidase activity in the lysates of transfected cells. In contrast to luciferase assays, no expensive equipment is needed to assay β-galactosidase activity. Cell lysates are simply incubated with a reaction buffer and ONPG substrate. β-Galactosidase converts the colorless ONPG substrate into galactose and the chromophore *o*-nitrophenol, yielding a bright yellow solution. The β-galactosidase activity of the solution can be quantitated using a spectrophotometer or a microplate reader to determine the amount of substrate converted at 420 nm.

The β-Galactosidase Assay Kit is excellent for determining enzyme activity in lysates from cells transfected with a β-galactosidase expression construct.[1,2] A detergent lysis step replaces the lengthy freeze-thaw step in the generation of cell lysates, greatly reducing the time to perform the assay.

PROTOCOL

Harvesting the Cells

1. Wash each 60-mm plate of cells twice with 5 ml of PBS buffer.
2. Add 1 ml of PBS buffer to each 60-mm plate.
3. Gently scrape down the cells with a Teflon® spatula and transfer the suspension into a 1.5-ml microcentrifuge tube.
4. Rinse the plate with 300 µl of PBS buffer and add the suspension to the microcentrifuge tube.
5. Pellet the cells at 12,000 × *g* for 1 minute at 4°C.
6. Remove the supernatant by aspiration and discard.
7. Mix the lysis buffer by gently shaking the container. Resuspend the cells in 200 µl of lysis buffer by pipetting the cell suspension up and down several times.
8. Incubate the cell suspension at room temperature for 5 minutes.
9. Spin the cell debris at 12,000 × *g* for 5 minutes at 4°C.
10. Transfer the supernatant into a fresh microcentrifuge tube and proceed to the *Macro* or *Micro β-Galactosidase Assay.*

Macro β-Galactosidase Assay

Note *Thaw the ONPG substrate and dissolve any precipitates before use.*

1. Pipet 50–100 µl of cell lysate into a 12 × 75-mm polystyrene tube and adjust the volume to 100 µl with ddH_2O.
2. Prepare 900 µl of buffer A–β-mercaptoethanol mixture by adding 45 µl of 1 M β-mercaptoethanol to 855 µl of buffer A. Mix the two components by inversion.
3. Add 900 µl of buffer A–β-mercaptoethanol mixture to the lysate and incubate the tube in a 37°C water bath for 5 minutes.
4. Add 200 µl of ONPG substrate that has been warmed to 37°C to the tube and vortex the tube for 5 seconds. Record the time of ONPG addition.

5. Incubate the reaction for 30 minutes or longer in a 37°C water bath until the sample turns bright yellow (the incubation period will vary).

   Note *If the solution turns bright yellow immediately, the cell lysate may contain too large a quantity of β-galactosidase for the quantity of ONPG in the reaction, resulting in an underestimate of the β-galactosidase activity. Repeat the reaction using less cell lysate and a longer incubation period at 37°C.*

6. Stop the reaction by adding 500 µl of stop solution and record the incubation period, which is the time expired between the addition of ONPG substrate and the addition of the stop solution.

7. Record the OD_{420} and determine specific activity and protein concentration (see *Appendix: Testing for β-Galactosidase Activity*).

   Notes *The samples should be read shortly after completion of the assay and should not be left for an extended period of time (e.g., overnight).*

   The optimal OD_{420} is 0.6–0.9.

Micro β-Galactosidase Assay

1. Pipet 15–50 µl of appropriately diluted cell lysate (~1:5) into a 96-well microtiter dish.

   Note *The volume in the wells should not exceed 300 µl.*

2. For each well, prepare 160 µl of buffer A–β-mercaptoethanol mixture by adding 8 µl of 1 M β-mercaptoethanol to 152 µl of buffer A. Mix the two components by inversion.

3. Add 110–145 µl of buffer A–β-mercaptoethanol mixture to each well for a final volume of 160 µl.

4. Cover and incubate the microtiter dish for 5 minutes at 37°C.

5. Add 50 µl of ONPG substrate to each well and cover the dish with a microtiter dish lid.

6. Incubate the dish in an incubator at 37°C until mixture turns bright yellow.

7. Terminate the reaction by adding 90 µl of stop solution and scan the microtiter dish in a microtiter dish reader set at 414–420 nm. Record the incubation period, which is the time expired between the addition of ONPG substrate and the addition of the stop solution.

8. To determine specific activity and protein concentration, refer to *Appendix: Testing for β-Galactosidase Activity*.

APPENDIX: TESTING FOR β-GALACTOSIDASE ACTIVITY

The β-galactosidase activity is calculated according to procedures outlined in reference 3. The modified formula therein expresses β-galactosidase activity as nmoles of β-galactose formed per minute per mg of lysate at 37°C.[3]

1. $OD_{420}/0.0045$ = nmoles formed per milliliter

2. nmoles/ml × total assay volume (lysate, buffer A, ONPG, and stop solution) = nmoles

3. nmoles/time of 37°C incubation = nmoles/minute (units)

4. Units/µl of lysate used in the assay/protein concentration of 1 µl lysate in milligrams = Units/mg of lysate

For accurate measurements of β-galactosidase activity, the amount of total protein in the cell lysate has to be determined using a kit for protein concentration determination. β-Galactosidase activity is expressed in units/mg of lysate.

TROUBLESHOOTING

Observation	Suggestion
No color development	Use only freshly prepared buffer A–β-mercaptoethanol mixture
	Check transfection conditions to ensure that β-galactosidase is being expressed by the cells
	Ensure that the incubation temperature is 37°C
	Incubate for a longer time (~3–4 hours) at 37°C
	Check pH of buffer A, which should be pH 7.5; optimal OD 420 is 0.6–0.9

PREPARATION OF MEDIA AND REAGENTS

Buffer A–β-Mercaptoethanol Mixture (pH 7.5)

Note *Prepare fresh before each assay.*

Buffer A
100 mM NaH_2PO_4
10 mM KCl
1 mM $MgSO_4$
50 mM β-Mercaptoethanol

o-Nitrophenyl-β-D-Galactopyranoside (ONPG)

4 mg/ml in 100 mM NaH_2PO_4 buffer (pH 7.5)

Stop Solution

1 M Na_2CO_3

REFERENCES

1. Spear, D., Edwards, P., Huvar, A. and Lernhardt, W. (1993) *Strategies* 6(1):10-12.
2. Huvar, A. and Lernhardt, W. (1994) *Strategies* 7(1):17-18.
3. Nielsen, D. A., Chou, J., MacKrell, A. J., Casadaban, M. J. and Steiner, D. F. (1983) *Proc Natl Acad Sci U S A* 80(17):5198-202.

ENDNOTES

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