# β-Gal Assay Kit

**Catalog no. K1455-01**

**Version F**

082301
*28-0102*

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# Important Information

## Storage and Shipping

The β-Gal Assay Kit is shipped on dry ice. Please see the table below for storage conditions. **Note:** All solutions may be stored at -20°C without affecting the performance of the reagent. If necessary, warm solutions to room temperature and mix well before use.

## Kit Components

The kit components, composition, amount and storage conditions are listed in the table below. Enough reagents are included for one hundred 800 µl reactions or four hundred 200 µl reactions (equivalent to four 96-well microtiter plates).

| Component | Composition | Amount | Storage |
|---|---|---|---|
| 10X PBS (Phosphate Buffered Saline) | 0.017 M $KH_2PO_4$<br>0.05 M $Na_2HPO_4$<br>1.5 M NaCl, pH 7.4 | 60 ml | Room temperature or +4°C |
| 1X Lysis Buffer | 0.25 M Tris, pH 8.0 | 10 ml | Room temperature or +4°C |
| 10X Cleavage Buffer | 0.6 M $Na_2HPO_4$-$7H_2O$<br>0.4 M $NaH_2PO_4$-$H_2O$<br>0.1 M KCl<br>0.01 M $MgSO_4$-$7H_2O$<br>pH 7 | 25 ml | +4°C |
| β-mercaptoethanol | 14.3 M | 750 µl | Room temperature or +4°C |
| ONPG | 4 mg/ml ONPG in sterile water | 5 x 2 ml | -20°C |
| Stop Buffer | 1 M Sodium Carbonate | 55 ml | Room temperature or +4°C |

## Product Qualification

The quality control of this kit is based on purified β-galactosidase from Sigma (Catalog no. G-6008). One to ten milliunits of β-galactosidase (0.001U/µl) were assayed in a total volume of 300 µl at 37°C as described in the manual and stopped after 10 minutes. The resulting OD values plotted against the amounts of purified β-galactosidase must result in a linear relationship. In addition, the $OD_{420}$ of the reaction containing 10 milliunits must be greater than 0.400

## Materials Supplied by the User

The following materials are required for use with this kit:

- 37°C water bath
- spectrophotometer capable of measuring 420 NM
- microcentrifuge/centrifuge
- plastic cuvettes
- microcentrifuge tubes

# Overview

## Introduction

The β-Gal Assay Kit from Invitrogen provides the reagents required to quickly measure the levels of active β-galactosidase expressed in cells transfected with plasmids expressing *lacZ*. *LacZ* is a bacterial gene often used as a reporter construct in eukaryotic transfection experiments because the gene product, β-galactosidase, is resistant to proteolysis in cellular lysates, and its activity is easily assayed. β-galactosidase catalyzes the hydrolysis of β-galactosides such as ortho-nitrophenyl-β-D-galactopyranoside (ONPG). Hydrolysis of ONPG to the ONP anion produces a bright yellow color with a peak absorbance at 420 nm that can be quantified using a spectrophotometer.

## Experimental Outline

The table below provides a general overview of the steps involved in the β-Gal Assay Kit.

| Step | Action |
|---|---|
| 1 | Transfect cells with a *lacZ* containing expression construct. |
| 2 | Harvest cells using trypsin/EDTA or by scraping. |
| 3 | Lyse cells by pelleting, resuspending in lysis buffer, and alternating freeze-thaw cycles. |
| 4 | Transfer soluble lysate to a fresh tube, dilute and assay for activity. |
| 5 | Calculate the units of β-galactosidase activity in the lysate and normalize to the amount of total protein. |


β-mercaptoethanol is highly toxic. **Wear gloves, lab coats and other protective gear when handling.**

**Important**

Please dispose of all hazardous substances in accordance with federal, state, and local regulations.

## Protocols

This manual includes three protocols:

| Protocol | Purpose | Page |
|---|---|---|
| β-galactosidase Assay | To **quantify** β-galactosidase activity in a small number of samples | 3 |
| Microtiter Plate Assay 1 | To **detect** the presence of β-galactosidase activity in any number of samples | 4 |
| Microtiter Plate Assay 2 | To **quantify** β-galactosidase activity in a large number of samples | 4 |

# β-galactosidase Assay

## Before Starting

Be sure to have cells transfected with a *lacZ* construct and prepare the following solutions:

- Dilute the 10X PBS and 10X Cleavage Buffer to make 1X solutions by adding 90 ml distilled, deionized water to 10 ml of stock solution.
- Add 270 µl β-mercaptoethanol to 100 ml 1X Cleavage Buffer before use.
- Unused 1X solutions may be stored at +4°C for 6 months for use in future assays.

## Sample Preparation

1. Starting with transfected cells, remove the growth medium from the cells and wash transfected cell monolayers once with 1X PBS.
2. Harvest cell monolayers with trypsin/EDTA or by scraping cells into 1 ml 1X PBS.
3. Centrifuge cells at 250 x g for 5 minutes. Aspirate the supernatant.
4. Completely resuspend the pellet in 1X Lysis Buffer. Keep sample at +4°C.

   Note: The amount of Lysis Buffer used varies depending on the size of the cell pellet. For a pellet harvested 48-72 hours posttransfection from a 60 mm plate, use 50 µl 1X Lysis Buffer. For a 100 mm plate, use 100 µl.
4. Freeze the sample on dry ice and thaw in a 37°C water bath. Repeat 2 times.
5. Pellet the insoluble cell material by centrifugation at maximum speed at +4°C for 5 minutes. Transfer the supernatant to a new microcentrifuge tube.

## β-galactosidase Assay

1. For each sample (see Step 5 above), take 1-10 µl of cell lysate and transfer to a fresh microcentrifuge tube.
2. Bring to a final volume of 30 µl with distilled, deionized water.
3. Add 70 µl of ONPG and 200 µl 1X Cleavage Buffer with β-mercaptoethanol. Mix by gently flicking the tube and centrifuging briefly.
4. Incubate the tube at 37°C for 30 minutes. You should see a faint yellow color develop if β-galactosidase is present.
5. To stop the reaction, add 500 µl of Stop Buffer. There may be some intensifying of the color. Final volume is 800 µl.
6. Read the absorbance at 420 nm against a blank containing ONPG and Cleavage Buffer without lysate. Be sure to assay a sample of the untransfected cell lysate as a control.
7. Assay at least three different volumes of lysate (i.e. 1, 5, and 10 µl). Changes in absorbance should be linear with respect to the amount of lysate assayed. If it is not, you will not get an accurate determination of activity.
8. Once you have obtained an accurate reading of your lysate, determine the protein concentration of the lysate, and calculate the specific activity of the lysate using the following formula:

Specific activity = nmoles of ONPG hydrolyzed/t/mg protein

$$\text{nmoles of ONPG hydrolyzed} = \frac{(OD_{420})\ (8 \times 10^5 \text{ nanoliters})}{(4500 \text{ nl/nmoles-cm})(1 \text{ cm})}$$

where 4500 is the extinction coefficient, t = the time of incubation in minutes at 37°C (i.e. 30 minutes), and mg protein is the amount of protein assayed, which can be determined using the BCA assay (Pierce Chemical). Be sure to subtract the background activity of the untransfected cell lysate.

# Microtiter Plate Assay

**Important**

To perform these assays, you will need a spectrophotometer that is capable of reading microtiter plates.

### Microtiter Plate Assay #1

1. Take a 96-well plate containing transfected cells and wash two times with 100 µl 1X PBS per well. Aspirate the final wash.
2. Add 10 µl of Lysis Buffer to each well.
3. Freeze the plate on dry ice and thaw in a 37°C water bath. Repeat 2 times.
4. Add 50 µl of 1X Cleavage Buffer with β-mercaptoethanol and 17 µl of ONPG to each well.
5. Cover plate and incubate at 37°C for 30 minutes. A faint yellow color should develop. Record incubation time.
6. Add 125 µl of Stop Buffer to each well. A yellow color indicates the presence of β-galactosidase. Final volume is 192 µl. See the formula below in Steps 5 and 6 to calculate the amount of β-galactosidase.

### Microtiter Plate Assay #2

1. Prepare cell lysates as in **Sample Preparation**, previous page.
2. Dilute sample 1:100 and add 1-10 µl to a well containing 50 µl 1X Cleavage Buffer with β-mercaptoethanol and 17 µl ONPG.
3. Cover plate and incubate at 37°C for 30 minutes. A faint yellow color should develop. Record incubation time.
4. Add 125 µl Stop Buffer to the reaction and read the absorbance at 420 nm using untransfected cells as the blank. Final volume is 192 µl.
5. Calculate the amount of ONPG hydrolyzed using the following formula:

$$\text{nmoles ONPG hydrolyzed} = \frac{(OD_{420})\,(1.92 \times 10^5\ \text{nl})}{(4500\ \text{nl/nmole-cm})\,(1\ \text{cm})}$$

6. To determine the specific activity of β-galactosidase (nmoles ONPG hydrolyzed per minute per mg protein), use the following formula.

$$\text{Specific activity} = \text{nmoles ONPG hydrolyzed}/t/\text{mg protein}$$

where t = the time of incubation in minutes at 37°C and mg protein is the amount of protein assayed. You will need to determine the protein concentration of the lysate. (This can be determined using a protein assay kit such as the BCA Assay by Pierce.) Be sure to subtract the background activity of the untransfected cell lysate.

# Troubleshooting Guide

## Problems and Solutions

The table below describes solutions to some possible problems.

| Problem | Cause | Solution |
|---|---|---|
| No color development | No ONPG added to reaction | Repeat assay and be sure to add ONPG. |
| | Low expression levels | Repeat assay using more lysate. |
| | | Poor transfection. Check transfection conditions and repeat transfection. |
| | Cells did not lyse during freeze/thaw procedure | Repeat freeze/thaw procedure on pellet. Vortex. |
| | Incorrect incubation temperature | Check that incubation temperature was at 37°C. |
| Color development is too intense | High expression levels or too much sample | Repeat assay with more dilute sample or incubate for a shorter period of time. **Note:** This will affect your calculations compared to other samples. |
| Good color development with low units of β-galactosidase | Incorrect calculations | Repeat calculations. Remember to factor in any dilutions made during the assay. |
| | Spectrophotometer is not set to read at 420 nm | Adjust spectrophotometer to read at 420 nm. |

# Technical Service

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**http://www.invitrogen.com**

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

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4. All requests will be faxed unless another method is selected.
5. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.

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# Technical Service, continued

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3E Company
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