# ONPG β-Galactosidase Assay Kit

## (Colorimetric Readout)



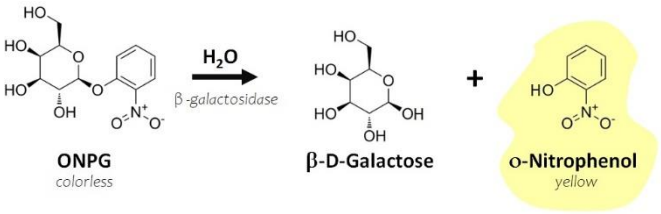
### Ordering info

TBK0543, 500 assays

### Description

ONPG β-Galactosidase Assay Kit is an extraordinary tool commonly used in molecular biology and microbiology research to measure the activity of beta-galactosidase enzyme. This assay can be used to detect the presence of certain bacterial strains and to monitor expression of *lac*Z as β galactosidase reporter gene.

ONPG β-Galactosidase Assay Kit is a quantitative method based on the cleavage of the synthetic chromogenic lactose analogous substrate ortho-nitrophenyl-beta-galactopyranoside (ONPG), releasing a bright yellow product called ortho-nitrophenol (ONP). The ONP production per unit time is directly proportional to the activity of β-galactosidase in the sample.




### Features

- Accurate, simple and reproducible assay, around 3,5-6% inter- and 3.3-5.5% intra- assay coefficient of variation.
- Narrow dynamic range, linearity from 0.2 to 2 nmol/min/mg protein.
- Colorimetric readout at 420 nm.
- Highly Sensitive, the assay can detect beta-galactosidase activity in the range of 0.1-10 nmol/min/mg protein.
- Proteolytic resistance of β-galactosidase, lysates could be assayed directly or store at -80 °C for at least 2 months.

### Kit Components

| Components | TBK0543 |
|---|---|
| BGA Lysis Solution [a] | 50 mL |
| 10x BGA Reaction Buffer [a] | 12 mL |
| BGA Stop Solution [a] | 70 mL |
| BME, β mercaptoethanol [a] | 500 µL |
| β galactosidase (0.4 U/ µL) [b] | 100 µL |
| ONPG Solution (4 mg/ mL) [b] | 10 mL |

*Order Info Kit Components: BGA Lysis Solution (TBB0570) | 10x BGA Reaction Buffer (TBB0571) | BGA Stop Solution (TBB0572) | Beta galactosidase (TBZ0330) | ONPG Solution (TBR0267) | BME (TBR0107).*

### Storage

[a] Store at 4 °C

[b] Store at -20 °C. Protect ONPG Solution from light.

The kit is shipped with blue ice.

### Unit Definition

One unit of β-Galactosidase hydrolyzes 1 micromole of ONPG to ONP and galactose per minute at pH 7.5 and 37 °C.

### Quality Control

The kit is tested in a functional assay.

### Applications

- Normalization of transfection efficiency in mammalian cells.
- Study of protein trafficking, strength of promoters and enhancer with β-galactosidase as reporter.
- Protein-protein interaction studies.

# PROTOCOL

## I. REAGENT PREPARATION

- 2x Reaction Buffer: Prepare enough buffer to your assay setup. For 100 reactions, dilute 1 mL 10x BGA Reaction Buffer with 4 mL Deionized Water. Add 17,5 µL β-mercaptoethanol. This buffer could be stored at 4 °C.

## II. CELL LYSATE PREPARATION

For transient expression, cell lysates must be prepared 48-72 hours after plasmid transfection. As negative control, prepare cell lysates from the same cell line that have not been transfected.

### *Suspension cells*

1. Centrifuge the cells (~ 2 mL) at 250 g, 5 minutes. Eliminate growth medium.
2. Wash twice the cells with PBS 1x (without $Ca^{2+}$ and $Mg^{2+}$). Discard the supernatant.
3. Add 50 µL BGA Lysis Solution and incubate at 37 °C, 30 minutes.

### *Adherent cells*

1. Discard growth medium and wash once the cells with PBS 1x (without $Ca^{2+}$ and $Mg^{2+}$). Discard PBS 1x.
2. Add a sufficient volume of BGA Lysis Solution to cover the cells. The next table could be indicative.

| Culture Format | BGA Lysis Solution per well |
|---|---|
| 96-well plate | 50 µL |
| 24-well plate | 250 µL |
| 12-well plate | 500 µL |
| 6-well plate | 800 µL |
| 60 mm dish | 1500 µL |

*If transfection efficiency is very high, it may be necessary to dilute the lysate in BGA Lysis Solution. On the contrary, if the expression is low, reduce the volume of BGA Lysis Solution.*

3. Incubate at room temperature for 15 minutes, swirling the plates several times to ensure an optimal lysis.

   *Optimal lysis is essential to obtain reproducible results.*
4. Transfer the cell lysate to a microcentrifuge tube by pipetting.
5. Spin the lysates at 4 °C for 2 minutes to eliminate cellular debris.
6. Transfer the supernatant to a microcentrifuge tube.

   *The lysates may be assayed directly or stored at -80 °C for at least 2 months.*

## III. ASSAY SETUP

All samples must run at least in duplicate, preferably triplicate. Keep the samples and standard on ice. Equilibrate at room temperature the rest of components.

- **Standard curve**: Firstly, prepare fresh **β galactosidase Solution** at 40 mU/ µL. Make 1:10 dilution of the provided enzyme, adding 10 µL β-galactosidase (0.4 U/ µL) to 90 µL BGA Lysis Solution. After that, in clean tubes, prepare a standard curve as follow:

| β -galactosidase Activity (U/µL) | 10 | 8 | 6 | 4 | 2 | 1 | 0.5 | 0 |
|---|---|---|---|---|---|---|---|---|
| β-galactosidase Solution 40 mU/ µL (µL) | 10 | 8 | 6 | 4 | 2 | 1 | 0.5 | 0 |
| BGA Lysis Solution (µL) | 30 | 32 | 34 | 36 | 38 | 39 | 39.5 | 40 |

Add 10 µL of each dilution to its corresponding wells of 96-well plate.

- **Negative Control**: Cells lacking β-galactosidase, such as LT2 or non-transfected cells.
- **Positive Control**: Cells with high β-galactosidase activity.
- **Plate setup**:

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 10 U/ µL | | | Sample 1 | | | Sample 8 | | | Sample 15 | | |
| **B** | 8 U/ µL | | | Sample 2 | | | Sample 9 | | | Sample 16 | | |
| **C** | 6 U/ µL | | | Sample 3 | | | Sample 10 | | | Sample 17 | | |
| **D** | 4 U/ µL | | | Sample 4 | | | Sample 11 | | | Sample 18 | | |
| **E** | 2 U/ µL | | | Sample 5 | | | Sample 12 | | | Sample 19 | | |
| **F** | 1 U/ µL | | | Sample 6 | | | Sample 13 | | | Sample 20 | | |
| **G** | 0.5 U/ µL | | | Sample 7 | | | Sample 14 | | | Sample 21 | | |
| **H** | 0 U/ µL | | | NEGATIVE CONTROL | | | POSITIVE CONTROL | | | Sample 22 | | |

STANDARD CURVE BLANK NEGATIVE CONTROL POSITIVE CONTROL SAMPLES

## IV. BETA GALACTOSIDASE ASSAY

1. Following the plate setup:
   - Add **10 µL** of dilution prepared for **Standard Curve**.
   - Add **10 µL Cell Lysate** from samples and from negative and positive control.
2. With a multichannel pipette, add **15 µL ONPG Solution** to each well.
3. With the multichannel pipette, add **50 µL 1x BGA Reaction Buffer** to each well.
4. Shake the plate carefully for 30 seconds.
5. Cover the plate with metallic film and incubate it at 37 °C for 30 minutes.
6. Add **125 µL BGA Stop Solution**
7. Read the absorbance at 420 nm.

## V. BETA GALACTOSIDASE ACTIVITY DETERMINATION

The absorbance from the blank control well is used as the background absorbance. Subtract the background absorbance from samples absorbance.

To quantify the amount of β-galactosidase in the samples, interpolate them on the enzyme standard curve.