

# **ab189815**

## **Beta Galactosidase Assay Kit (LacZ, Blue)**

Instructions for use:

For the quantitative measurement of beta-Galactosidase activity in live cells by flow cytometry.

View kit datasheet: [www.abcam.com/ab189815](http://www.abcam.com/ab189815)

(use [www.abcam.cn/ab189815](http://www.abcam.cn/ab189815) for China, or  
[www.abcam.co.jp/ab189815](http://www.abcam.co.jp/ab189815) for Japan)

This product is for research use only and is not intended for diagnostic use.

# Table of Contents

---

<b>INTRODUCTION</b>	<b>1</b>
<b>1. BACKGROUND</b>	<b>1</b>
<b>2. ASSAY SUMMARY – FLOW</b>	<b>2</b>
<b>3. ASSAY SUMMARY – MICROPLATE</b>	<b>3</b>
<b>GENERAL INFORMATION</b>	<b>4</b>
<b>4. PRECAUTIONS</b>	<b>4</b>
<b>5. STORAGE AND STABILITY</b>	<b>4</b>
<b>6. LIMITATIONS</b>	<b>5</b>
<b>7. MATERIALS SUPPLIED</b>	<b>5</b>
<b>8. MATERIALS REQUIRED, NOT SUPPLIED</b>	<b>6</b>
<b>9. TECHNICAL HINTS</b>	<b>7</b>
<b>ASSAY PREPARATION</b>	<b>8</b>
<b>10. REAGENT PREPARATION</b>	<b>8</b>
<b>ASSAY PROCEDURE</b>	<b>10</b>
<b>11. ASSAY PROCEDURE – FLOW CYTOMETRY</b>	<b>10</b>
<b>12. ASSAY PROCEDURE –MICROPLATE READER</b>	<b>12</b>
<b>DATA ANALYSIS</b>	<b>14</b>
<b>13. CALCULATIONS</b>	<b>14</b>
<b>RESOURCES</b>	<b>15</b>
<b>14. FAQs</b>	<b>15</b>
<b>15. NOTES</b>	<b>16</b>

## 1. BACKGROUND

Beta Galactosidase Assay Kit (LacZ, Blue) (ab189815) is an assay based of the fluorogenic substrate 3-carboxyumbelliferyl- $\beta$ -D-galactopyranoside (CUG), a blue dye (Ex/Em = 390/460 nm) that, when analyzed by flow cytometry, has been shown to be several orders of magnitude more sensitive than chromogenic assays based on X-Gal detection.

In addition, the high water solubility and detection limits of CUG substrate makes this assay easy to adapt to automated ELISA-type systems.

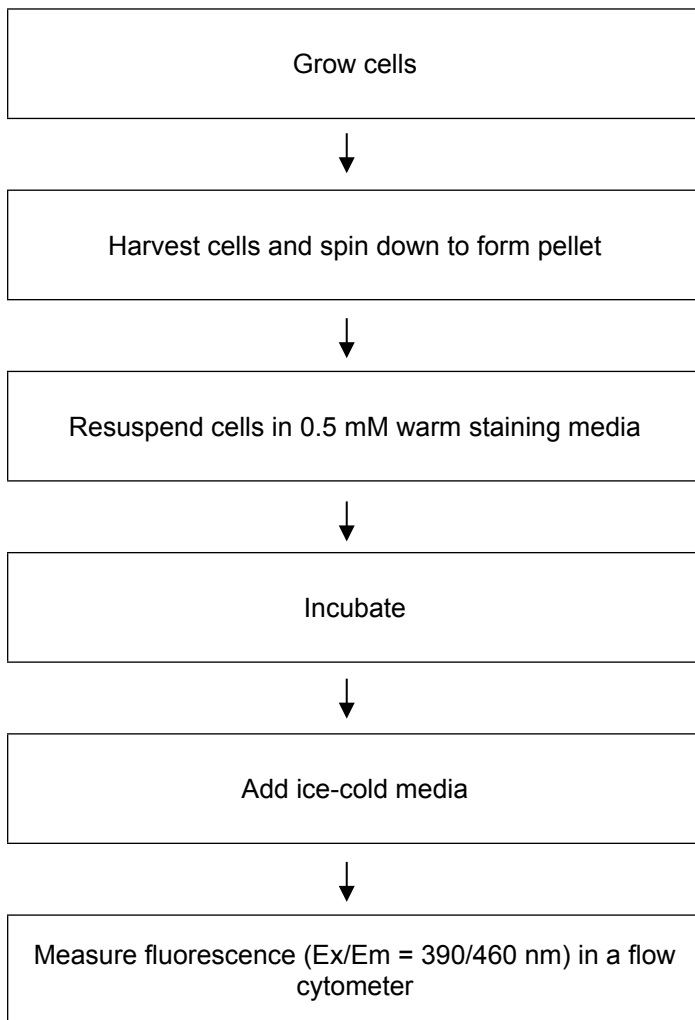
This assay has been especially designed for dual-labeling experiments where the cells to be analyzed have been labeled with a fluorescein-based probe such as FITC-labeled antibody.

This detection kit provides reagents to assay 50 to 500 microwells (using 10 or 1 mM substrate reagent) on 96-well microtiter plates. Lower concentrations of substrate (1 – 5 mM) are routinely used for lower enzyme concentrations.

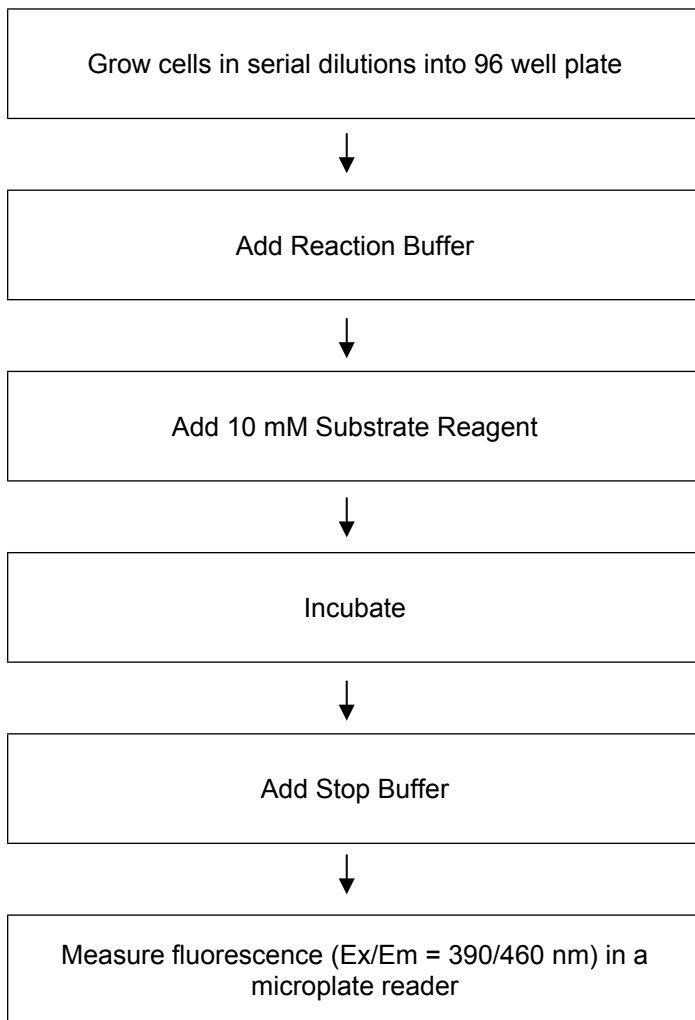
We also provide a quick test assay (30 minutes) for qualitative analysis of *lacZ* activity in cultured cells.

One of the most common reporter genes used in molecular biology applications is the *E.coli lacZ* gene that codes for an active subunit of  $\beta$ -galactosidase *in vivo*. Since the enzyme is generally absent in normal mammalian, yeast, some bacterial and even plant cells, it can be detected at very low levels. Monitoring *lacZ* expression, as well as co-expressed genes or promoter efficiency, has become a routine technique. FACS analysis assays can detect as few as 5 copies of  $\beta$ -galactosidase per cell.

## 2. ASSAY SUMMARY – FLOW



### 3. ASSAY SUMMARY – MICROPLATE



### 4. PRECAUTIONS

**Please read these instructions carefully prior to beginning the assay.**

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

### 5. STORAGE AND STABILITY

**Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

## GENERAL INFORMATION

### 6. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

### 7. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Substrate Reagent (50 mM)	500 $\mu$ L	-20°C	-20°C
Reference Standard (20 $\mu$ M)	500 $\mu$ L	-20°C	-20°C
Inhibitor	1 mL	-20°C	-20°C

### 8. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorometric microplate reader or flow cytometer capable of measuring fluorescence at Ex/Em = 390/460 nm
- MilliQ water or other type of double distilled water (ddH<sub>2</sub>O)
- General tissue culture supplies
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- PBS
- Tubes for the preparation of reagents and buffer solutions
- Sterile, tissue culture treated, 96 well plate with clear flat bottom, preferably black
- Purified  $\beta$ -galactosidase enzyme – for microplate assay only.

To prepare Reaction Buffer:

- 200 mM Na<sub>2</sub>HPO<sub>4</sub> (sodium phosphate, dibasic solution)
- 200 mM NaH<sub>2</sub>PO<sub>4</sub> (sodium phosphate, monobasic solution)
- 1 mM MgCl<sub>2</sub>
- 10 mM  $\beta$ -mercaptoethanol:  $\beta$ -mercaptoethanol is potentially dangerous, so please use in a well ventilated area or fume hood.
- 0.1% Triton X-100

To prepare Stop Buffer:

- 500 mM Glycine
- EDTA
- 10N NaOH

### 9. TECHNICAL HINTS

- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice. Clear bottom, dark sided microplates are recommended with this assay. Clear sided microplates have not been tested with this kit.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

### 10. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening

#### 10.1. **Substrate Reagent (50 mM Stock in ddH<sub>2</sub>O) for microplate assay:**

Dilute stock solution in 2 mL of Reaction Buffer (Step 10.4) to make a working concentration of 10 mM. Aliquot working Substrate Reagent so that you have enough volume to perform the desired number of assays. Avoid freeze/thaw cycles. Store at -20°C. Diluted Substrate Reagent is stable for at least 6 months. Keep on ice while in use.

**NOTE:** This dilution step is for the microplate assay ONLY.

#### 10.2. **Reference Standard (20 mM Stock in absolute methanol) – for microplate assay ONLY:**

Dilute stock solution in Stop Buffer (Step 10.5) to the highest concentration of the substrate reagent to be tested – this will provide the highest possible fluorescent signal of the dye (typical initial dilution: 1:50). Aliquot diluted Reference Standard so that you have enough volume to perform the desired number of assays. Avoid freeze/thaw cycles. Store at -20°C. Diluted Standard is stable for at least 6 months. Keep on ice while in use.

#### 10.3. **Inhibitor (30 mM Stock in ddH<sub>2</sub>O):**

Ready to use as supplied. Aliquot Inhibitor so that you have enough volume to perform the desired number of assays. Avoid freeze/thaw cycles. Store at -20°C. Keep on ice while in use.

### 10.4. Reaction Buffer (NOT PROVIDED):

- 10.4.1. Prepare 50 mL of 200 mM Sodium Phosphate Buffer (2X), pH 7.0 by mixing 30.5 mL of 200 mM  $\text{Na}_2\text{HPO}_4$  with 19.5 mL of 200 mM  $\text{NaH}_2\text{PO}_4$ .
- 10.4.2. Check pH, and adjust to pH 7.0 as needed with small volumes of the monobasic or dibasic stock solutions.
- 10.4.3. Add 1 mL of 100mM  $\text{MgCl}_2$ , 1ml 1M  $\beta$ -mercaptoethanol and 1ml 10% Triton X-100 to the Sodium Phosphate Buffer.
- 10.4.4. Dilute to a final volume of 100 mL with  $\text{ddH}_2\text{O}$ .

### 10.5. Stop Buffer (NOT PROVIDED):

- 10.5.1. Dilute 500 mM glycine and 10 mM EDTA in  $\text{ddH}_2\text{O}$ .
- 10.5.2. Adjust to pH 12.0 with 10 N NaOH.
- 10.5.3. Adjust final volume to 75 mL with  $\text{ddH}_2\text{O}$ .

### 11. ASSAY PROCEDURE – FLOW CYTOMETRY

- Equilibrate all materials and prepared reagents to correct temperature prior to use.
- We recommended to assay all controls and samples in duplicate.
- Prepare all reagents and samples as directed in the previous sections.

#### 11.1. Grow cells expressing the *lacZ* gene so that they are in exponential growth phase on the day of the experiment.

**NOTE:** *Unhealthy cells or those grown to confluence often exhibit high background staining due to endogenous galactosidase activity or low pH conditions.*

#### 11.2. Harvest cells as required.

- 11.2.1. Suspension cells: collect cells in a conical tube and wash by centrifugation once in PBS. Spin down cells to a weak pellet.
- 11.2.2. Adherent cells: wash cells once in PBS. Trypsinize as per standard protocols, and collect cells in a conical tube. Spin down cells to a weak pellet.

#### 11.3. Staining procedure:

- 11.3.1. Warm the cell pellet in a water bath at 37°C for 10 minutes.
- 11.3.2. Prepare the staining media by diluting the Substrate Reagent to between 200  $\mu$ M to 2 mM concentration (i.e. 1:12500 to 1:25, respectively) with ice-cold media (non-serum), then warming to 37°C.
- 11.3.3. Add an equal volume of pre-warmed Staining media to the cell pellet – typically 100 – 200  $\mu$ L of Substrate Reagent (100  $\mu$ M to 2 mM concentration) to 100 – 200  $\mu$ L of cell pellet.

**NOTE:** *prepare parallel background controls by adding 100  $\mu$ L of substrate reagent to a tube containing 100  $\mu$ L of ddH<sub>2</sub>O or media.*

- 11.3.4. Mix the cell suspension (and controls) thoroughly and rapidly.
- 11.3.5. Incubate at 37°C in a water bath for 20 – 25 minutes.

## ASSAY PROCEDURE

**NOTE:** Longer incubation periods may be used with appropriate control to guard against high background fluorescence from decomposition at these temperatures.

- 11.3.6. Terminate dye loading by adding 1.8 mL of ice-cold media containing serum to the cells and controls. Keep stained cells on ice until analysis.

### 11.4. **Beta-Gal detection:**

- 11.4.1. Analyze cells and background control on a flow cytometer. Establish forward and side scatter gates to exclude debris and cellular aggregates from analysis.
- 11.4.2. Read fluorescence at  $E_m = 460 \text{ nm}$ , using an appropriate excitation filter for excitation at  $E_x = 390 \text{ nm}$ .

**NOTE:** we recommend to adjust concentration range of the fluorescent probe for optimum signal and sensitivity. Previous studies indicate that the labelling of cells is virtually independent of the initial fluorescent probe concentration when within the range of  $100 \text{ pM} - 2 \text{ mM}$ .

Staining is somewhat time dependent, therefore a time course for the experiments should be generated for initial trials.

### 12. ASSAY PROCEDURE – MICROPLATE READER

- Equilibrate all materials and prepared reagents to correct temperature prior to use.
- We recommended to assay all controls and samples in duplicate.
- Prepare all reagents and samples as directed in the previous sections.

#### 12.1. Sample preparation:

- 12.1.1. Prepare samples from cells expressing the *lacZ* gene as per your experimental design requirements. Samples can be cell lysates, purified enzyme or cell suspension.
- 12.1.2. Calibration curve: set up a calibration curve using known concentrations of purified  $\beta$ -galactosidase enzyme, making sure you cover the range of the analyte. Typical working range of assay = 1 – 1000 picograms.

#### 12.2. Plate loading:

Sample = 20 – 50  $\mu$ L sample (adjust volume to 50  $\mu$ L/well with Reaction Buffer).

Blank = 20 – 50  $\mu$ L Reaction Buffer (adjust volume to 50  $\mu$ L/well with Reaction Buffer).

Reference Standard = 50  $\mu$ L diluted Standard (step 10.2).

Calibration curve = 50  $\mu$ L sequential concentrations of purified  $\beta$ -galactosidase enzyme.

#### 12.3. Set up reaction:

- 12.3.1. Add 100  $\mu$ L of Reaction Buffer to each well. Mix thoroughly and incubate for 5 minutes.
- 12.3.2. Add 50  $\mu$ L of 10 mM Substrate Reagent to each well. Mix thoroughly. **NOTE:** *Lower concentrations of Substrate Reagent are routinely used for lower enzyme concentrations.*
- 12.3.3. Incubate for 20 minutes at room temperature.

## ASSAY PROCEDURE

**NOTE:** If lower substrate concentrations are used, incubation times may need to be adjusted proportionally.

- 12.3.4. Add 100  $\mu$ L Stop Buffer to each well. Incubate for 10 minutes before reading plate.

**NOTE:** Plate readings can be at any time up to 3 hours after stopping the reaction. Store unread plates at 4°C covered with parafilm or plastic wrap if they are not to be read immediately.

### 12.4. Beta-Gal detection:

- 12.4.1. Measure plate on a fluorescence plate reader at Ex/Em= 390/460 nm in end point mode. Use Reference Standard for optimizing spectrometer conditions (recommended initial dilution: 1:50).

## QUICK TEST ASSAY – MICROPLATE READER

The following procedure is a quick assay that can be used to determine if cells show *lacZ* activity, for example to check for transfection efficiency or to determine whether *lacZ* is expressed in the cells. The assay can be performed after routine subculturing of cell lines with as little as 5,000 cells. This quick test assay is not quantitative.

- 12.5. Pipette 150  $\mu$ L of sample cell suspension into individual microtiter wells. Include wells for blanks with 150  $\mu$ L of fresh media (no cells).
- 12.6. Add 50  $\mu$ L of 10 mM (or lower) Substrate Reagent to each well. Mix thoroughly.
- 12.7. Incubate for 20 minutes at room temperature.
- 12.8. Measure plate on a fluorescence plate reader at Ex/Em = 390/460 nm in a kinetic mode, every 5 – 10 minutes, from at least 20 min – 2 hours at room temperature.
- 12.9. Calculate signal:
  - 12.9.1. Subtract fluorescence from the blank wells from each sample wells.
  - 12.9.2. Average readings of duplicate samples.
  - 12.9.3. Plot graph of fluorescence increase over time.

### 13. CALCULATIONS

- Microplate assay ONLY: samples producing signals higher than calibration curve should be further diluted in appropriate buffer and reanalysed, then multiply the concentration found in well by the appropriate dilution factor.
- 13.1. Subtract fluorescence of the blanks from the sample and/or calibration curve readings.
  - 13.2. Average the duplicate reading for each sample and/or calibration curve.
  - 13.3. Plot the corrected fluorescence values for each concentration of the purified  $\beta$ -galactosidase (log-log).
  - 13.4. Draw the best smooth curve through these points to construct the standard curve. Calculate the trend line equation based on your calibration curve data (use the equation that provides the most accurate fit).
  - 13.5. Extrapolate this data to determine the concentration of the enzyme in the original cell/tissue suspension.

### 14. FAQs

#### **How long will the Substrate Reagent remain stable?**

CUG should be stable in solution for at least several hours and even several days, if kept cold. Acidic or basic buffers will hasten the decomposition. Evidence of decomposition can be monitored by running a blank (non-*lacZ* cells) at the beginning and end of your assays.

#### **Is it possible to put the CU labeled cells in culture, or is the labeling reaction lethal?**

The CUG and the carboxyumbelliferone (fluorescent product) are not toxic, and you should be able to re-culture your cells after sorting. Keep in mind though that some cell toxicity may occur just because of the many manipulations in the FACS sorting, but the staining should not affect viability more than other type of staining protocol.

#### **What can I do if I have high levels of endogenous $\beta$ -galactosidase activity in my flow analysis?**

In case high levels of endogenous  $\beta$ -galactosidase are noticed,  $10^7$  cells/mL should be resuspended in media (with serum) containing 300  $\mu$ M Inhibitor. After incubating at 37°C for 20 minutes, centrifuge cells and proceed with staining procedure as per Step 11.3.3.

### 15. NOTES

## RESOURCES

## **Technical Support**

Copyright © 2025 Abcam, All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

**For all technical or commercial enquiries please go to:**

[www.abcam.com/contactus](http://www.abcam.com/contactus)

[www.abcam.cn/contactus](http://www.abcam.cn/contactus) (China)

[www.abcam.co.jp/contactus](http://www.abcam.co.jp/contactus) (Japan)