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ProductInformation

β-GALACTOSIDASE REPORTER GENE ACTIVITY DETECTION KIT

Product Number **GAL-A**

TECHNICAL BULLETIN

Product Description

Reporter genes are "markers" widely used for analysis of mutationally altered genes as well as gene regulation. The expressed reporter genes are detected by biochemical activity assays, by immunological analysis, or by histochemical staining of tissue sections or cells.

The β-galactosidase gene from *E. coli*, LacZ, is often used as a reporter gene in eukaryotic transfection. β-Galactosidase catalyzes the hydrolysis of various β-galactosides. Substrates designed to produce chromogenic, fluorescent, or chemiluminescent products are used for β-galactosidase activity detection. This kit utilizes o-nitrophenyl β-D-galactopyranoside (ONPG), a widely used chromogenic substrate for determination of β-galactosidase activity in transfected cells or tissues.

This kit provides all components needed for detection of β-galactosidase activity by a colorimetric assay. The colorless substrate, ONPG, is hydrolyzed by β-galactosidase to produce yellow colored o-nitrophenol. o-Nitrophenol absorbs light at 420 nm with the molar extinction of 4600 at a basic pH.

Unit definition: One unit is the amount of β-galactosidase that hydrolyses 1 μmol ONPG per minute at 37 °C.

Reagents

The kit is sufficient for 65 standard tests or 200 tests using 96-well microtiter plates.

- 5x Lysis Buffer, Product Code L 5784 — 25 ml
  250 mM HEPES, pH 7.5, 25 mM CHAPS
- 2X Assay Buffer, Product Code G 4663 — 10 ml
  200 mM sodium phosphate buffer, pH 7.3, 2 mM $MgCl_2$, 100 mM β-mercapto-ethanol,1.33 mg/ml o-nitrophenyl β-D-galactopyranoside (ONPG)
- Stop Solution, Product Code S 3680 — 35 ml
  1 M sodium carbonate
- β-Galactosidase Control Enzyme, Product Code G 4538, from *E. coli* — 100 units

Reagents and Equipment Required but not Provided

37 °C water bath
Spectrophotometer and/or ELISA reader with 420 nm filter
Dulbecco's Phosphate Buffered Saline (DPBS), Product Code D 8662
Calcium Phosphate Transfection Kit, Product Code CA-PHOS (optional)
ESCORT™, Product Code E 9770 (optional)

Reagent Preparation

Thaw all kit components. Mix each component well before use. Make sure that the 2x Assay Buffer is completely in solution.
Dilute 5x Lysis Buffer 1:5 with 0.2 μm filtered water to prepare 1x Lysis Buffer.
Place the 2x Assay Buffer and 1x Lysis Buffer on ice.

Precautions and Disclaimer

Sigma's β-Galactosidase Reporter Gene Activity Detection Kit is for laboratory use only. Not for drug, household, or other uses.

Storage/Stability

Store kit at –20 °C

Procedure

All steps must be performed in a laminar flow hood.

A. Transfect the cells with β-galactosidase encoding plasmid using Calcium Phosphate transfection kit (Product Code CA-PHOS) or ESCORT transfection reagent (Product Code E 9770). For control, transfect cells without DNA.
   Note: The efficiency of the transfection is largely dependent on the construct and quality of the DNA preparation. The duration of the transfection must be optimized by the researcher.

B. Analyze the β-galactosidase expression 40-72 hr post-transfection as follows:
   1. Aspirate the medium from the plates.
   2. Wash the cells three times with 1 ml DPBS.
   3. Add 200 µl of 1x lysis buffer to 3.5 cm plate or 600 µl to 6 cm plate.
   4. Incubate at room temperature for 15 minutes.
   5. Collect lysate in microcentrifuge tubes.
   6. Spin in a microcentrifuge at maximal speed for 5 minutes to remove any cell debris.
   7. Keep lysate on ice.
   8. Pipette a volume of 10 to 150 µl of transfected cell lysate and of control cell lysate into microcentrifuge tubes. The exact volume is dependent on the efficiency of the transformation and must be optimized.
   9. Complete the volume to 150 µl with 1x lysis buffer.
   10. Prepare the reagent blank by pipetting 150 µl 1x lysis buffer into a microcentrifuge tube.
   11. Add 150 µl 2x assay buffer to each microcentrifuge tube. Mix gently by vortexing.
   12. Incubate at 37 °C for 30 minutes or until a yellow color develops (t_{min}).
   13. Stop the reaction with 500 µl stop solution. Mix gently by vortexing. All reactions must be stopped after the same length of incubation.
   14. Read OD at 420 nm.
   15. Calculate the β-galactosidase activity in the sample as follows:

$$\text{Units/sample} = \frac{\text{OD} \times 0.8^*}{4.6 \times t_{min}}$$

   * Final reaction volume (ml)

   Note: OD values higher than 2 or lower than 0.1 are not accurate.

   16. Calculate the activity per plate

$$\text{Units/plate} = \text{Units/sample} \times \frac{\text{lysis volume}}{\text{sample volume}}$$

Control enzyme

The activity of the control enzyme is approx. 1 U/µl. Since the activity detection using this colorimetric assay is in the mUnit range the control enzyme has to be diluted. The test should be performed with 2-5 mU/ reaction.

1. Dilute control β-galactosidase enzyme 1:10,000 in 1X lysis buffer to obtain activity at the range of 0.1 mU/µl. Prepare three serial dilutions:

   a. 10 µl to 100 µl (x10)
   b. 10 µl Solution a to 100 µl (x100)
   c. 10 µl Solution b to 1000 µl (x10,000)

2. Pipette 20 µl and 40 µl of diluted enzyme Solution c in two microcentrifuge tubes.
3. Complete the volume in each tube to 150 µl with 1x lysis buffer. Mix gently by vortexing.
4. Prepare the reagent blank by pipetting 150 µl 1x lysis buffer into a microcentrifuge tube.
5. Add 150 µl 2x assay buffer to each microcentrifuge tube. Mix gently by vortexing.
6. Incubate at 37 °C for 30 minutes (t_{min}).
7. Stop the reaction with 500 µl stop solution. Mix gently by vortexing.
8. Read OD at 420 nm.
9. Calculate units of β-galactosidase

$$\text{Units/sample} = \frac{\text{OD} \times 0.8^*}{4.6 \times t_{min}}$$

   * Final reaction volume (ml)

10. Calculate the activity of the control enzyme (units/ml):

$$\text{Units/ml} = \text{Units/sample} \times \frac{10{,}000}{\text{sample vol., ml}}$$

Procedure using 96-well microtiter plate

1. Prepare cell lysate as described in section B, steps 1-7.
2. Pipette up to 50 µl of transfected cell lysate and of control cell lysate into microtiter plate wells. The exact volume is dependent on the efficiency of the transformation and must be optimized.
3. Complete volume of each level to 50 µl with 1x lysis buffer.
4. Pipette 50 µl of 1x lysis buffer in another well (blank).
5. Add 50 µl 2x assay buffer to each microtiter plate well. Mix by pipetting.
6. Incubate at 37 °C for 30 minutes or until a yellow color develops (t_{min}).
7. Add 150 µl stop solution. Mix by pipetting. All reactions must be stopped after the same length of incubation.

8. Read OD at 420 nm. Note: If this wavelength is not available, a close wavelength (for example, 405 nm) may be used although the absolute values will be lower.

Note: When calculating the β-galactosidase activity the calculation must also take into account the optical path of the light beam, the final assay volume and the lysate volume. The absorbance of 1 μmol/ml (1 mM) is 4.6 for an optical path of 1 cm.

Reference

1. Sambrook, J., et al., *Molecular Cloning: A Laboratory Manual*, Second edition, p.p. 1.86, B14 (Cold Spring Harbor Laboratory Press, Plainview, New York, 1989).

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