Senescence-Associated β-Galactosidase Staining Kit

Item No. 602010

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GENERAL INFORMATION

Materials Supplied

Item Number	Item Name	Quantity/Size	Storage Temperature
400448	X-Gal Reagent	1 vial/150 mg	-20°C
400447	Fixative Solution (10X)	1 vial/15 ml	-20°C
400439	Staining Buffer (10X)	1 vial/15 ml	-20°C
400445	Staining Supplement (100X)	1 vial/1.5 ml	-20°C
400446	Buffer Supplement (10X)	1 vial/15 ml	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. 37°C incubator
2. Phosphate buffered saline (PBS)
3. Tissue culture plate
4. DMSO or dimethyl formamide (DMF)
5. A source of senescent cells
6. Microscope
7. A source of pure water; glass-distilled, deionized, or ultrapure water is acceptable.
8. Hydrochloric acid (HCl)
9. Sodium hydroxide (NaOH)

INTRODUCTION

Background

Cellular senescence is a cell fate characterized by stable, irreversible cell cycle arrest and resistance to apoptosis.[1-5] There are two major pathways of cellular senescence, replicative and stress-induced, which induce senescence through various signaling pathways that converge and terminate at p53, p21, p16, or the tumor suppressor RB.[1,4] Replicative senescence is initiated by replication-induced telomere shortening, which is recognized by cells as a type of endogenous DNA damage. External or internal stressors such as oxidative stress, chemotherapy, radiation, or traumatic injury, induce the accumulation of damaged DNA and stress-induced cellular senescence. Markers of cellular senescence include lack of cell proliferation, large and flat morphology *in vitro*, lack of response to growth factors, the production of senescence-associated secretory factors (SASPs), and increased expression of the lysosomal and pH-dependent enzyme β-glucosidase.[2,3] Senescent cell burden is increased with aging and obesity, and is associated with the promotion of various diseases including, muscular dystrophy, osteoporosis, diabetes, age-related cataracts, neurodegeneration, and atherosclerosis.[1,5] The induction of senescence suppresses tumor initiation, however, persistent tumor cell senescence and the production of SASPs can induce senescence escape and tumor promotion.[1]

About This Assay

Cayman's Senescence-Associated β-Galactosidase Staining Kit provides a simple and fast method for the detection of senescence in cultured cells. After incubation with the staining solution at 37°C, a blue color develops in senescent cells, while non-senescent cells remain unstained. A sufficient amount of each reagent is provided to test 150 wells in a 12-well format.

Principle Of This Assay

Upon induction of senescence, cells undergo numerous phenotypical changes, including vacuolization, size enlargement, and altered gene expression. Additionally, senescent cells exhibit increased expression of the lysosomal and pH-dependent enzyme β-galactosidase. Upon incubation of senescent cells at an acidic pH, β-galactosidase cleaves the supplied colorless substrate into an insoluble chromogenic product, which can be used to visually discriminate between senescent and non-senescent cells.

PRE-ASSAY PREPARATION

Buffer Preparation

Staining Buffer (1X)

Dilute the Staining Buffer (10X) (Item No. 400439) 1:10 in pure water.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Thaw reagents using a water bath set to 37°C. Continue heating until no precipitates remain.

Fixative Solution (1X)

Dilute Fixative Solution (10X) (Item No. 400447) 1:10 in PBS. Keep on ice until use.

Staining Buffer (1X)

Dilute Staining Buffer (10X) (Item No. 400439) 1:10 in pure water.

X-Gal Solution

Dissolve 20 mg of X-Gal Reagent (Item No. 400448) in 1 mL of DMSO or DMF to prepare a 20X X-Gal solution. Excess X-Gal solution (20X) can be stored at -20°C for up to one month protected from light. Always use polypropylene or glass to make and store the X-Gal solution. Do not use polystyrene.

Cell Staining Solution

While cells are fixing, prepare the cell staining solution using a polypropylene tube. Prepare only enough cell staining solution for the number of wells that are to be stained. The protocol below yields enough cell staining solution for one well of a 12-well plate. Scale accordingly if using a different size plate (*e.g.*, doubling the volumes for a 6-well plate) or more than one well. For each well, combine:

420 µl Staining Buffer (1X)

50 µl Buffer Supplement (10X) (Item No. 400446)

5 µl Staining Supplement (100X) (Item No. 400445)

25 µl X-Gal solution (20X)

Test the pH of the cell staining solution to ensure it has a final pH of 5.9-6.1. If necessary, use HCl or NaOH to lower or raise the pH, respectively. *NOTE: pH variation can alter staining results, with low pH and high pH resulting in false positives and false negatives, respectively.*

Plate Set Up

Plate set up will depend on the specific experimental requirements of the end user. It is recommended to include one well as a negative staining control. For this well, incubate cells with cell staining solution in which the X-Gal has been omitted and replaced by 25 µl of DMSO or DMF.

Performing the Assay

The following protocol is designed for a single well in a 12-well plate. Scale accordingly if using a different size plate (*e.g.*, doubling the volumes for a 6-well plate) or more than one well. Methods to induce senescence are highly cell type dependent, and can't be broadly applied.

1. Plate cells and treat according to experimental design.
2. Remove culture medium from well and wash cells twice with 1 ml of 1X PBS.
3. Fix the cells with 500 µl of Fixative Solution (1X) for 10-15 minutes at room temperature. *NOTE: Fixative Solution is toxic and should be handled with care to avoid inhalation or exposure to bare skin.*
4. While cells are fixing, prepare sufficient cell staining solution for each well.
5. Remove fixative and wash cells twice with 1X PBS. Alternatively, cells can be left in 1X PBS, covered, and stored at 4°C overnight.
6. Carefully aspirate PBS from well. Add 500 µl of cell staining solution to each well. Cover the plate and incubate at 37°C for one hour up to overnight. Carbon dioxide levels in general 37°C incubators will alter the pH of the cell staining solution and affect color development. If an incubator without carbon dioxide is not available, we suggest placing the plate in a resealable zipper bag to help limit any effect of the carbon dioxide.
7. Observe cells under a microscope for the development of blue color.
8. For long-term storage of stained plates, remove cell staining solution and overlay cells with 70% glycerol. Store plates at 4°C.

ANALYSIS

Performance Characteristics

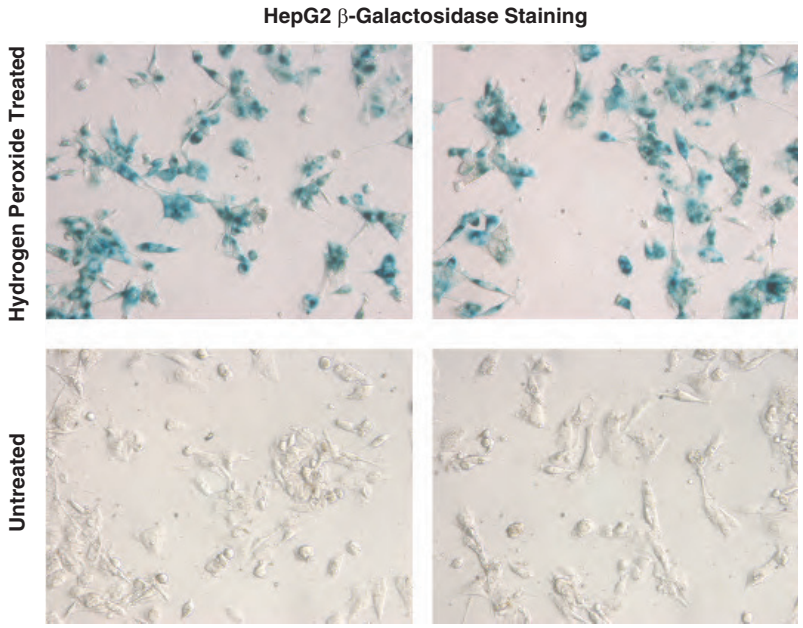

Figure 1. β-Galactosidase staining in HepG2 cells. HepG2 cells were seeded at 75,000 cells/well in a 12-well plate and incubated with either 1X PBS or 500 µM hydrogen peroxide for one hour. Cells were then washed and allowed to recover for five days. After five days, cells were stained for β-Galactosidase activity as described.

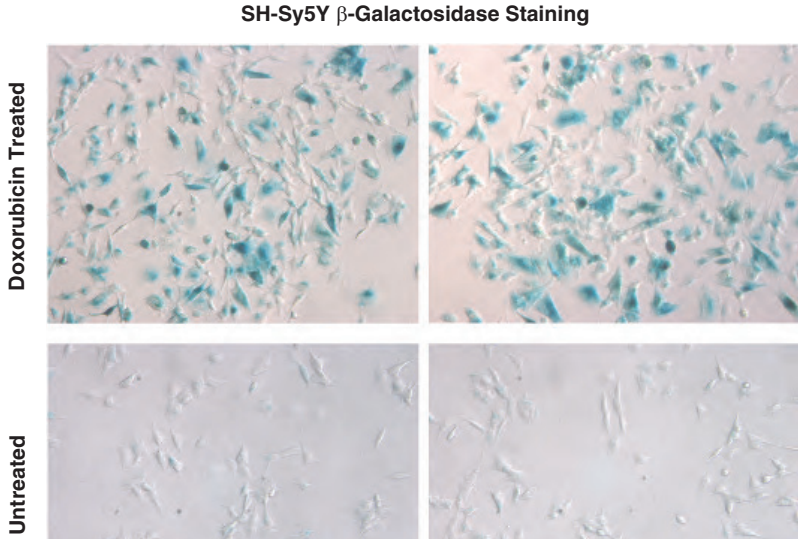

Figure 2. β-Galactosidase Staining in SH-SY5Y cells. SH-SY5Y cells were seeded at 50,000 cells/well in a 12-well plate and treated with either vehicle or 250 nM doxorubicin for 24 hours. Cells were then washed and allowed to recover for one week. After one week, cells were stained for β-Galactosidase activity as described.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Poor color formation	A. pH of cell staining solution was altered due to carbon dioxide B. Insufficient incubation time	Incubate for a longer period of time and/or in a carbon dioxide-free incubator
Precipitates in Staining Buffer (10X) or Staining Supplement (100X)	Precipitation due to temperature changes	Warm to 37°C using a water bath to dissolve precipitates
Crystal formation after incubation	Solvent evaporation	Keep plate sealed during overnight incubation to limit evaporation

References

1. Beck, J., Horikawa, I., and Harris, C. Cellular senescence: Mechanisms, morphology, and mouse models. *Vet. Pathol.* **57(6)**, 747-757 (2020).

2. Zhou, D., Borsa, M., and Simon, A.K. Hallmarks and detection techniques of cellular senescence and cellular ageing in immune cells. *Aging Cell* **20**, e13316.
3. Salama, R., Sadaie, M., Hoare, M., *et al.* Cellular senescence and its effector programs. *Genes Dev.* **28(2)**, 99-114 (2014).
4. Muñoz-Espín, D. and Serrano, M. Cellular senescence: From physiology to pathology. *Nat. Rev. Mol. Cell Biol.* **15(7)**, 482-496 (2014).
5. Palmer, A.K., Gustafson, B., Kirkland, J.L. *et al.* Cellu ar senescence: At the nexus between ageing and diabetes. *Diabetologia* **62(10)**, 1835-1841 (2019).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.