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)	2 vials/9 ml	-20°C
401016	Staining Solution A (5X)	2 vials/16 ml	-20°C
401017	Staining Solution B (50X)	2 vials/1.6 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.

The Fixative Solution (10X) (Item No. 400447) is toxic and a known carcinogen. Wear appropriate personal protective equipment (*e.g.* safety glasses, gloves, and a lab coat) and work in a ventilated area when using this reagent. For more information, review the Safety Data Sheet.

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
5. A source of senescent cells
6. Microscope
7. A source of pure water; glass-distilled, deionized, or ultrapure water is acceptable.

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. 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. A sufficient amount of each reagent is provided to test 150 wells in a 12-well format.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **X-Gal Reagent - (Item No. 400448)**

   This vial contains 150 mg of X-gal. In a polypropylene or glass tube, prepare a 20 mg/ml X-gal working solution by dissolving 20 mg in 1 ml of DMSO. This is a sufficient volume to prepare 20 ml of the Cell Staining Solution (see **Preparation of Assay-Specific Reagents**, page 8). Scale as needed. If not using all once, aliquot and store the solution at -20°C where it will be stable for one month if protected from light. *NOTE: Always use polypropylene or glass tubes when preparing the X-gal working solution. Do not use polystyrene.*

2. **Fixative Solution (10X) - (Item No. 400447)**

   Each vial contains 9 ml of Fixative Solution (10X). Thaw in a water bath set to 37°C. Gently invert the vial to mix and continue heating until no visible precipitates remain. Dilute to 1X by mixing 1 ml with 9 ml of PBS and keep on ice. This is a sufficient volume to assay one plate. Scale as needed. *NOTE: Fixative is carcinogenic and toxic if inhaled. Use necessary precautions to avoid inhalation or exposure to skin.*

3. **Staining Solution A (5X) - (Item No. 401016)**

   Each vial contains 16 ml of Staining Solution A (5X). Thaw at room temperature and vortex briefly to mix contents. Once thawed and mixed, it is ready to use to prepare the Cell Staining Solution. If not using all at once, aliquot remaining reagent and store at -20°C where it will be stable for 6 months.

4. **Staining Solution B (50X) - (Item No. 401017)**

   Each vial contains 1.6 ml of Staining Solution B (50X). Thaw at 37°C and vortex briefly to dissolve contents. Once completely dissolved, it is ready to use to prepare the Cell Staining Solution. If not using all at once, aliquot remaining reagent and store at -20°C where it will be stable for 6 months.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Cell Staining Solution

The Cell Staining and Control Solutions should be freshly prepared in polypropylene tubes while cells are fixing (see **Performing the Assay** step 3, page 11). Combine reagents following Table 1 below. This yields a sufficient volume of staining solutions for a typical 12-well plate experiment. If using a more than one plate or a different sized plate, consult Table 2 on page 9 and scale as needed.

Reagent	Cell Staining Solution	Control Solution (Optional)
Staining Solution A (5X)	1,500 µl	600 µl
Staining Solution B (50X)	150 µl	60 µl
X-Gal Solution, 20 mg/ml	375 µl	--
DMSO	--	150 µl
Pure water	5,475 µl	2,190 µl
Final Volume:	**7,500 µl**	**3,000 µl**

Table 1. Preparation of staining solutions

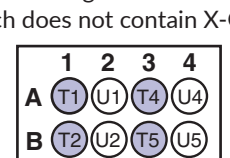
Plate Type	Volume of Cell Staining/Control Solution Per Well (µl)
6-Well	1,000
12-Well	500
24-Well	250
96-Well	100

Table 2. Recommended volumes for different plate types

It is *strongly recommended* to sterile filter these solutions prior to use to minimize the formation of precipitates in the wells.

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout is shown in Figure 1. However, the plate setup will depend on the specific experimental requirements of the end user. It is recommended to include at least one well to serve as a negative control wherein cells are incubated with the Control Solution, which does not contain X-Gal.



T1-T5 = Treated Wells
U1-U5 = Untreated Wells
NC = Negative Control Wells

Figure 1. Sample plate layout

Performing the Assay

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

1. Plate cells and induce senescence according to experimental design.
2. Gently aspirate culture medium and wash cells twice with 1 ml of 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. Prepare the **Cell Staining and Control Solutions** (see page 8).
5. Gently aspirate fixative and wash cells twice with PBS. Alternatively, cells can be left in PBS, covered, and stored at 4°C overnight.
6. Gently aspirate PBS and add 500 µl of Cell Staining or Control Solution to the appropriate wells.
7. Cover and incubate in a 37°C dry incubator. *NOTE: High carbon dioxide levels will alter the pH of the Cell Staining Solution and affect color development. If a dry incubator is not available, place plate in a resealable zipper bag to minimize exposure to carbon dioxide.*
8. Cells may be examined under a microscope for the development of blue color after 4 hours. The color may continue to develop for up to 16 hours.
9. For long-term storage of stained plates, gently aspirate and replace the liquid by overlaying cells with 70% glycerol. Store plates at 4°C.

ANALYSIS

Performance Characteristics

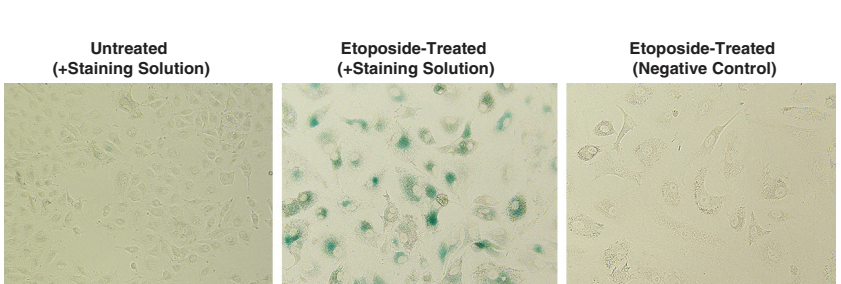


Figure 2. β-Galactosidase staining in A549 cells. A549 cells were seeded at 4,000 cells per well in a 96-well plate and incubated with DMSO (untreated) or 10 µM etoposide for 48 hours. Cells were washed and allowed to recover for 5 days prior to staining for β-galactosidase activity. Scale bar: 200 µm.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Poor color formation	A. pH of cell staining solution was altered due to carbon dioxide B. Insufficient staining time	Incubate for a longer period of time and/or in a carbon dioxide-free incubator
Precipitates in Fixative Solution (10X) or Staining Solution B (50X)	Precipitation due to temperature changes	Warm to 37°C using a water bath to dissolve precipitates
Crystal formation after incubation	A. Solvent evaporation B. X-gal precipitate formation	A. Keep plate covered during overnight incubation to limit evaporation B. Sterile filter Cell Staining Solution before use

References

1. Beck, J., Horikawa, I., and Harris, C. *Vet. Pathol.* **57(6)**, 747-757 (2020).
2. Zhou, D., Borsa, M., and Simon, A.K. *Aging Cell* **20**, e13316.
3. Salama, R., Sadaie, M., Hoare, M., *et al. Genes Dev.* **28(2)**, 99-114 (2014).
4. Muñoz-Espín, D. and Serrano, M. *Nat. Rev. Mol. Cell Biol.* **15(7)**, 482-496 (2014).
5. Palmer, A.K., Gustafson, B., Kirkland, J.L. *et al. Diabetologia* **62(10)**, 1835-1841 (2019).

NOTES

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