

Cellular Senescence Assay Kit

KAA002

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USA & Canada

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Introduction

Cellular senescence is one of the most fundamental aspects of cell behavior, and is thought to play a critical role in regulating cellular lifespan both *in vitro* and *in vivo* [1-3]. Primary somatic cells grown *in vitro* do not proliferate indefinitely. Instead, after a period of rapid proliferation, cell division rate slows, and ultimately ceases altogether, with the cells becoming unresponsive to mitogenic stimuli. This process is termed cellular senescence, and senescent cells have a well-defined accompanying phenotype - increased cell size, distinctive flat morphology, accumulated lipofuscin granules, wide changes in gene expression, and activity of senescence-associated β -galactosidase (SA- β -gal) [2,3].

It is generally believed that cellular senescence reflects some of the changes that occur during the aging of organisms, and senescent cells have been detected *in vivo* at sites of age-related pathology, such as benign hyperplastic prostate [4] and atherosclerotic lesions [5]. Recent studies have also provided convincing demonstrations of cellular senescence occurring *in vivo* in response to internally-and externally-induced stress signals [6,7]. In all of these studies, senescence was characterized by the appearance of senescence-associated β -galactosidase (SA- β -gal) activity, in common with the senescent phenotype *in vitro*.

Cellular senescence has become an increasingly important target in the development of novel therapeutics. Emerging data implicates senescence bypass in the development of cancer and suggests that senescence may represent a tumor suppressor mechanism. The demonstration that tumor cells can be induced to undergo replicative senescence following the introduction of negative cell-cycle regulators, anti-telomerase peptides, or drug treatment suggests that induction of senescence can be exploited as a basis for cancer therapy [8,9].

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Test Principle

As outlined above, a classic characteristic of the senescent phenotype is the induction of senescence-associated $\beta\text{-galactosidase}$ (SA- $\beta\text{-gal}$) activity. SA- $\beta\text{-gal}$ is present only in senescent cells, not in presenescent, quiescent, or proliferating cells. Chemicon's Cellular Senescence Assay Kit provides all the reagents required to efficiently detect SA- β -gal activity at pH 6.0 in cultured cells and tissue sections. In this assay, SA- β -gal catalyzes the hydrolysis of X-gal, which results in the accumulation of a distinctive blue color in senescent cells. Each kit provides sufficient quantities of reagents to perform at least 50 assays in 35 mm wells.

Kit Components

- 1. 100X Fixing Solution: (Part No. 2004755) One 1.5 mL vial
- 2. 10X Staining Solution A: (Part No. 2004756) One 15 mL bottle
- 3. <u>10X Staining Solution B</u>: (Part No. 2004754) One 15 mL bottle
- 4. X-gal Solution: (Part No. 2004752) Two 1.5 mL vials

Materials Not Supplied

- 1. 1X PBS (Phosphate Buffered Saline)
- 2. 37°C incubator
- 3. Phase contrast or light microscope
- 4. Polypropylene tubes
- 5. Cells / Tissue samples
- 6. 70% glycerol (optional)

Precautions

• Please refer to the Material Safety Data Sheet at www.chemicon.com for any necessary precautions.

Storage

Store X-gal solution protected from light at -20°C, and other kit components at 4°C. All components supplied are stable for 1 year.

Assay Instructions

The following protocol is designed for one 35mm well of a 6-well plate.

Set-up:

- 1. Prepare a 1X PBS solution (not provided).
- 2. Dilute the 100X Fixing Solution with PBS to make a 1X working solution. 1mL fixing solution /well is sufficient (e.g. Add 10 μ L fixing solution to 990 μ L PBS).
- 3. Prepare SA-β-gal Detection Solution by mixing Staining Solutions A and B, X-Gal and PBS as indicated in Table 1. Prepare fresh for each use. See Table 1 for suggested volumes. Mix well before use.

Table 1. Preparation of SA-β-gal Detection Solution

Reagents	1 well	5 wells	10 wells
	(35 mm)	(35 mm)	(35 mm)
Staining Solution A (10X)	200 μL	1 mL	2 mL
Staining Solution B (10X)	200 μL	1 mL	2 mL
X-Gal	50 μL	250 μL	500 μL
PBS	1.55 mL	7.75 mL	15.5 mL
Total	2 mL	10 mL	20 mL

Procedure:

- 1. Aspirate the growth medium from the cells.
- 2. Wash the cells once with 2 mL 1X PBS and aspirate the wash.
- Add 1 mL 1X Fixing Solution per well. Incubate at room temperature for 10 - 15 minutes.
- 4. Remove the Fixing Solution and wash the cells twice with 2 mL 1X PBS. Aspirate after each wash.
- 5. Add 2 mL of freshly prepared 1X SA-β-gal Detection Solution.
- 6. Incubate the cells at 37°C, without CO₂ and protected from the light, for at least 4 hours. Samples may safely be left overnight.
- Remove the SA-β-gal Detection Solution. Wash the stained cells twice with 2mL PBS. Aspirate after each wash.
- 8. Count the blue stained cells under phase contrast, or light microscopy.
- 9. For long term storage, overlay the stained cells with 70% glycerol diluted in 1X PBS. Store at 4 8°C.

Sample Results

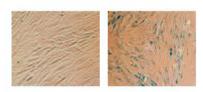


Figure 1. SA-β-gal staining of proliferating (left) and senescent (right) WI-38 human fibroblasts, using CHEMICON®'s Cellular Senescence Assay Kit

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KAA002

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