Cellular Senescence Assay Kit

KAA002

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Not for use in diagnostic procedures.

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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 β-galactosidase (SA-β-gal) activity. SA-β-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. 10X Staining Solution B: (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 -20ºC, and other kit components at 4ºC. 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 µL fixing solution to 990 µL 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 (35 mm)**	**5 wells (35 mm)**	**10 wells (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.
3. 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 CO_2 and protected from the light, for at least 4 hours. Samples may safely be left overnight.
7. 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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