NOTE: Revisions to
"Preparation of Reagents"

Product Manual

Quantitative Cellular Senescence Assay Kit (SA-β-gal, Fluorometric)

Catalog Number

CBA-232	10 assays (35 mm dishes)
CBA-232-5	5 x 10 assays (35 mm dishes)

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Normal primary cells proliferate in culture for a limited number of population doublings prior to undergoing terminal growth arrest and acquiring a senescent phenotype. This finite life span correlates with the age of the organism and with the life expectancy of the species from which the cells were obtained; such that the older the age or the shorter the life span, the less the ability of the cells to undergo population doubling. Senescent cells are characterized by an irreversible G_1 growth arrest involving the repression of genes that drive cell cycle progression and the upregulation of cell cycle inhibitors like $p16^{INK4a}$, p53, and its transcriptional target, $p21^{CIP1}$. They are resistant to mitogen-induced proliferation, and assume a characteristic enlarged, flattened morphology. Research into the pathways that positively regulate senescence and ways cells bypass senescence is therefore critical in understanding carcinogenesis. Normal cells have several mechanisms in place to protect against uncontrolled proliferation and tumorigenesis.

Senescent cells show common biochemical markers such as expression of an acidic senescence-associated ß-galactosidase (SA-ß-Gal) activity. While senescence has been characterized primarily in cultured cells, there is also evidence that it occurs in vivo. Cells expressing markers of senescence such as SA-ß-Gal have been identified in normal tissues.

Our Quantitative Cellular Senescence Assay Kit uses a fluorogenic substrate to measure SA-ß-Gal activity. This compound is a membrane permeable, non-fluorescent substrate of ß-galactosidase, which after hydrolysis of the galactosyl residues emits green fluorescence and remains confined within the cell. In comparison to the conventional cytochemical assay using X-gal, the use of a fluorogenic substrate greatly enhanced the sensitivity of the assay. Also, the non-toxic fluorogenic substrate allows you to study living cells by flow cytometry for more quantitative measurement of SA-ß-Gal activity. Each kit provides sufficient quantities to perform up to 10 assays in 35 mm dishes.

Related Products

1. CBA-230: Cellular Senescence Assay Kit (SA-β-gal Staining)
2. CBA-231: 96-Well Cellular Senescence Assay Kit (SA-β-gal Activity, Fluorometric Format)
3. CBA-240: CytoSelect™ Cell Viability and Cytotoxicity Assay
4. AKR-100: β Galactosidase Staining Kit

Kit Components (shipped on blue ice)

1. Cell Pretreatment Solution (1000X) (Part No. 123201): One 25 µL tube
2. SA-ß-Gal Substrate (200X) (Part No. 123202): One 100 µL amber tube

Materials Not Supplied

1. PBS
2. 37ºC Cell Culture Incubator
3. Light microscope
4. Senescent cells or tissue samples

Storage

Aliquot all kit components as needed to avoid repeated freeze-thaw cycles and store at -20ºC.

Preparation of Reagents

- 1X Cell Pretreatment Solution: Prepare desired amount of 1X Cell Pretreatment Solution by diluting the provided 1000X stock 1:1000 in culture medium. For example, add 4 µL of 1000X Cell Pretreatment Solution to 4.0 mL of culture medium. Use the diluted cell pretreatment solution within 4 hrs.

Assay Protocol (35 mm dish)

1. Aspirate the medium from the senescent cells expressing SA-ß-Gal and add 2 mL of 1X Cell Pretreatment Solution. Incubate at 37ºC for 2 hrs.
2. Add 10 µL of 200X SA-ß-Gal Substrate Solution directly to the cells in 1X Cell Pretreatment Solution. Gently mix and incubate at 37ºC for 4 hrs to overnight.
3. Wash the stained cells three times with 3 mL of 1X PBS.
4. Analyze the senescent cells by one of the following methods
    a. Flow cytometer (only after cells are trypsinized and washed in cold PBS containing 2% FBS).
    b. Epifluorescence microscope (Excitation: 485 nm/Emission: 520 nm)

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Recent Product Citations

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