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Product Manual

# 96-Well Cellular Senescence Assay Kit (SA- $\beta$ -gal Activity, Fluorometric Format), Trial Size

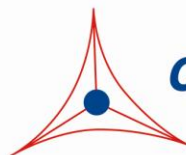
Catalog Number

CBA-231-T

24 assays

**FOR RESEARCH USE ONLY**  
Not for use in diagnostic procedures

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## **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<sub>1</sub> growth arrest involving the repression of genes that drive cell cycle progression and the upregulation of cell cycle inhibitors like p16<sup>INK4a</sup>, p53, and its transcriptional target, p21<sup>CIP1</sup>. 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  $\beta$ -galactosidase (SA- $\beta$ -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- $\beta$ -Gal have been identified in normal tissues.

The 96-well Cellular Senescence Assay Kit provides an easy-to-use and efficient method to determine the cellular senescence by measuring SA- $\beta$ -Gal activity using a fluorometric substrate. This quantitative assay uses cell lysate for both SA- $\beta$ -galactosidase activity determination and normalization of samples containing different cell numbers. Each Trial Size kit provides sufficient quantities to perform up to 24 assays in a 96-well plate.

## **Related Products**

1. CBA-080: CytoSelect™ 24-Well Anoikis Assay
2. CBA-081: CytoSelect™ 96-Well Anoikis Assay
3. CBA-230: Cellular Senescence Assay Kit (SA- $\beta$ -gal Staining)
4. CBA-232: Quantitative Cellular Senescence Assay (SA  $\beta$ -Gal)
5. CBA-240: CytoSelect™ Cell Viability and Cytotoxicity Assay
6. AKR-100:  $\beta$  Galactosidase Staining Kit

## **Kit Components**

1. 2X Cell Lysis Buffer (Part No. 123101-T): One 2 mL tube
2. 2X Reaction Buffer (Part No. 123102-T): One 2 mL tube
3. SA- $\beta$ -Gal Substrate (20X) (Part No. 123103-T): One 75  $\mu$ L amber tube
4. Stop Solution (Part No. 123104-T): Three 2 mL tubes

## **Materials Not Supplied**

1. Senescent cells or tissue samples
2. 37°C Incubator
3.  $\beta$ -mercaptoethanol
4. 96-well plate suitable for a fluorescence plate reader
5. 96-well Fluorometer
6. Protein Assay Reagents

## **Storage**

Store SA- $\beta$ -gal substrate solution protected from light at -20°C. Store all other components at room temperature.

## **Preparation of Reagents**

- 1X Cell Lysis Buffer: Prepare a 1X Cell Lysis Buffer by diluting the provided 2X stock 1:2 in ddH<sub>2</sub>O. Store the diluted solution at room temperature for up to six months. Immediately before use, add proper amount of proteinase inhibitors such as PMSF.
- 2X Assay Buffer: Immediately before use, add  $\beta$ -mercaptoethanol to 2X Reaction Buffer at a final concentration of 10 mM and dilute 20X SA- $\beta$ -Gal Substrate to 1X with 2X Reaction Buffer containing 10 mM  $\beta$ -mercaptoethanol. Don't store 2X Assay Buffer.

<b>Reagent</b>	<b>96-well</b>	<b>24-well</b>	<b>6-well</b>	<b>10 cm</b>
1X Cell Lysis Buffer	100 $\mu$ L	400 $\mu$ L	1000 $\mu$ L	1500 $\mu$ L

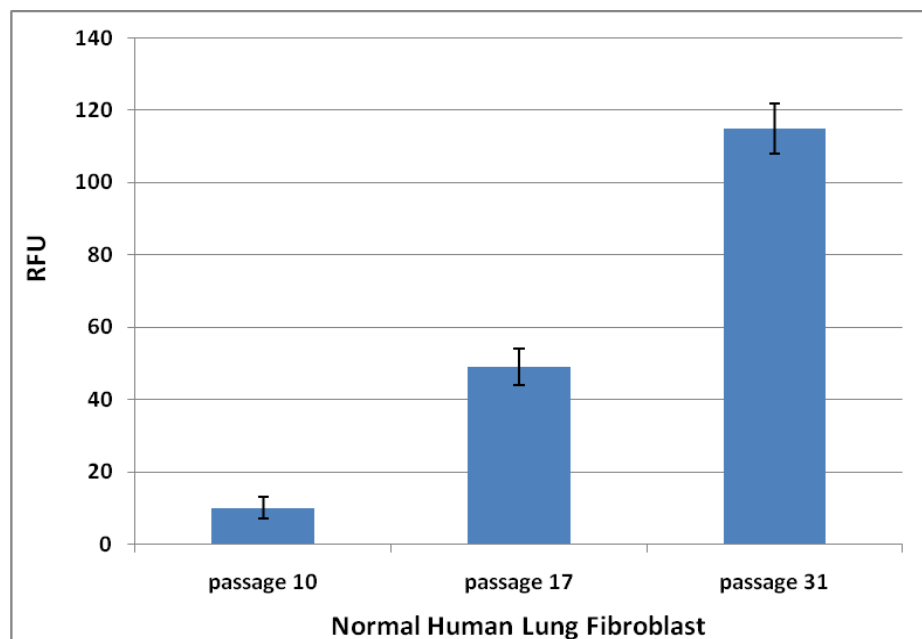
**Table 1. 1X Cell Lysis Buffer Required per Well for Various Culture Plates.**

## **Assay Protocol**

1. Aspirate the medium from the senescent cells.
2. Wash the cells once with 200  $\mu$ L of cold 1X PBS and aspirate the wash.
3. Add 100  $\mu$ L of cold 1X Cell Lysis Buffer (see the table above for the required amount of 1X Cell Lysis Buffer of other plate formats). Incubate at 4°C for 5 minutes. Transfer the whole lysate to a microcentrifuge tube and centrifuge 10 minutes at 4°C. Collect supernatant as cell lysate.
4. (optional) Determine the total protein concentration of each cell lysate sample by protein assay such as Pierce's BCA protein Assay.
5. Transfer 50  $\mu$ L of the cell lysate to a 96-well plate. Add 50  $\mu$ L of freshly prepared 2X Assay Buffer. Incubate the wells at 37°C protected from light for 1- 3 hr.
6. Remove 50  $\mu$ L of the reaction mixture to a 96-well plate suitable for fluorescence measurement. Stop the reaction by adding 200  $\mu$ L of Stop solution.
7. Read fluorescence with a fluorescence plate reader at 360 nm (Excitation) / 465 nm (Emission).

## **Example of Results**

The following figures demonstrate typical with the 96-well Cellular Senescence Assay Kit. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 355 nm/460 nm filter set. One should use the data below for reference only. This data should not be used to interpret actual results.



**Figure 1: SA-β-Gal activity in Senescent Human Lung Fibroblast HFL-1 Cells.** Normal HFL-1 cells with different passage numbers were lysed. Lysates were allowed to incubate with SA-β-Gal Substrate for 1 hr at 37°C. SA-β-Gal activities were measured as described in the Assay Protocol.

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