Product Manual

96-Well Cellular Senescence Assay Kit (SA-β-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_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.

The 96-well Cellular Senescence Assay Kit provides an easy-to-use and efficient method to determine the cellular senescence by measuring SA-ß-Gal activity using a fluorometric substrate. This quantitative assay uses cell lysate for both SA-β-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-β-gal Staining)
4. CBA-232: Quantitative Cellular Senescence Assay (SA β-Gal)
5. CBA-240: CytoSelect™ Cell Viability and Cytotoxicity Assay
6. AKR-100: β 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-ß-Gal Substrate (20X) (Part No. 123103-T): One 75 µL amber tube
4. Stop Solution (Part No. 123104-T): Three 2 mL tubes

Materials Not Supplied

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

Storage

Store SA-β-gal substrate solution protected from light at -20ºC. 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_2O. 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 β-mercaptoethanol to 2X Reaction Buffer at a final concentration of 10 mM and dilute 20X SA-ß-Gal Substrate to 1X with 2X Reaction Buffer containing 10 mM β-mercaptoethanol . Don't store 2X Assay Buffer.

Reagent	96-well	24-well	6-well	10 cm
1X Cell Lysis Buffer	100 µL	400 µL	1000 µL	1500 µL

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 µL of cold 1X PBS and aspirate the wash.
3. Add 100 µL 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 4ºC for 5 minutes. Transfer the whole lysate to a microcentrifuge tube and centrifuge 10 minutes at 4ºC. 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 µL of the cell lysate to a 96-well plate. Add 50 µL of freshly prepared 2X Assay Buffer. Incubate the wells at 37ºC protected from light for 1- 3 hr.
6. Remove 50 µL of the reaction mixture to a 96-well plate suitable for fluorescence measurement. Stop the reaction by adding 200 µL 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.

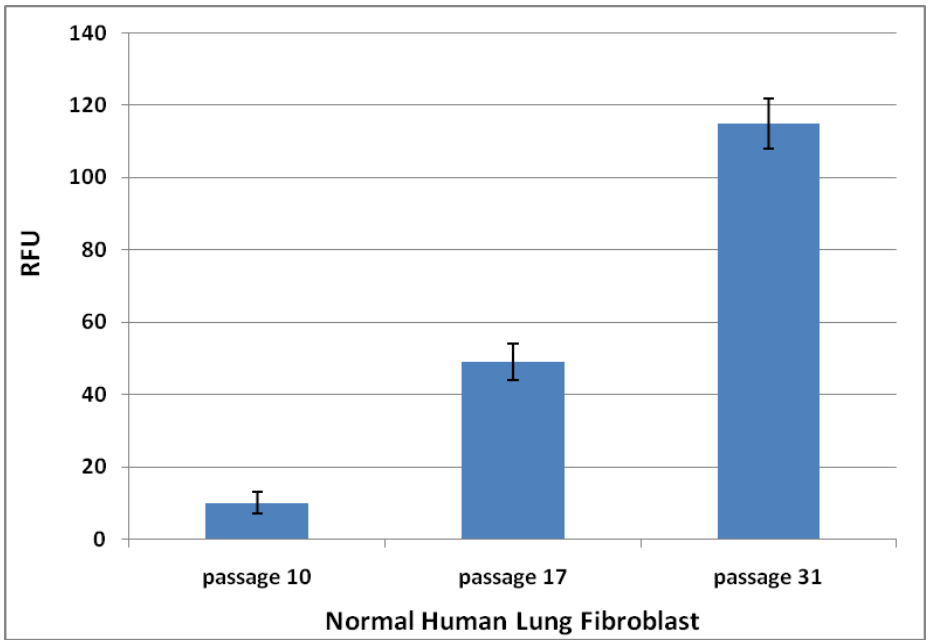

Figure 1: SA-ß-Gal activity in Senescent Human Lung Fibroblast HFL-1 Cells. Normal HFL-1 cells with different passage numbers were lysed. Lysates were allowed to incubate with SA-ß-Gal Substrate for 1 hr at 37ºC. SA-ß-Gal activities were measured as described in the Assay Protocol.

References

1. Current Protocols in Molecular Biology, John Wiley & Sons Press.
2. Campisi, J. (2000) *In Vivo* 14, 183-188.
3. Dimri, G. P., X. Lee, G. Basile, M. Acosta, G. Scott, C. Roskelley, E. E. Medrano, M. Linskens, I. Rubelj, O. Pereira-Smith, M. Peacocke, and J. Campisi. (1995) *Proc. Natl. Acad. Sci. USA* 92:9363-9367.

Recent Product Citation

1. Malhotra, D. et al. (2010). Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. *Nucleic Acids Res.* 10.1093/nar/gkq212.

Warranty

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