

**NOTE: Revisions to
“Preparation of Reagents”**

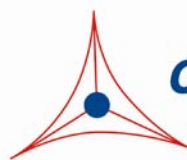
Product Manual

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 G₁ 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 β -galactosidase (SA- β -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- β -Gal have been identified in normal tissues.

Our Quantitative Cellular Senescence Assay Kit uses a fluorogenic substrate to measure SA- β -Gal activity. This compound is a membrane permeable, non-fluorescent substrate of β -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- β -Gal activity. Each kit provides sufficient quantities to perform up to 10 assays in 35 mm wells.

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

Kit Components

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

Materials Not Supplied

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

Storage

Aliquot and store all components at -20°C.

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-β-Gal and add 2 mL of 1X Cell Pretreatment Solution. Incubate at 37°C for 2 hrs.
2. Add 10 µL of 200X SA-β-Gal Substrate Solution directly to the cells in 1X Cell Pretreatment Solution. Gently mix and incubate at 37°C 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 after cells are trypsinized and washed in cold PBS containing 2% FBS.
 - b. Epifluorescence microscope (Excitation: 485 nm/Emission: 520 nm)

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 Citations

1. Won, Y.H. et al. (2016). Elucidation of relevant neuroinflammation mechanisms using gene expression profiling in patients with amyotrophic lateral sclerosis. *PLoS One* 11:e0165290.
2. Chae, S. Y. et al. (2016). Gardenia jasminoides extract-capped gold nanoparticles reverse hydrogen peroxide-induced premature senescence. *J. Photochem Photobiol B*. doi:10.1016/j.jphotobiol.2016.09.033.

3. Hu, W. et al. (2015). Mechanistic investigation of bone marrow suppression associated with palbociclib and its differentiation from cytotoxic chemotherapies. *Clin Cancer Res.* doi:10.1158/1078-0432.CCR-15-1421.
4. Kim, J. et al. (2014) p53 induces skin aging by depleting Blimp1+ sebaceous gland cells. *Cell Death Dis.* **5**: e1141
5. Grasso, D. et al. (2014). Genetic inactivation of the pancreatitis-inducible gene Nupr1 impairs PanIN formation by modulating KRas(G12D)-induced senescence. *Cell Death Differ.* **21**:1633-1641.
6. Landowski, T. H. et al. (2014). Targeting integrin $\alpha 6$ stimulates curative-type bone metastasis lesions in a xenograft model. *Mol Cancer Ther.* **13**:1558-1566.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2010-2017: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.