Product Manual

96-Well Cellular Senescence Assay Kit (SA-β-gal Activity, Fluorometric Format)

Catalog Number

CBA-231 **96 assays**

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 bypa**ss** 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 kit provides sufficient quantities to perform up to 90 assays in a 96-well plate.

Related Products

1. CBA-230: Cellular Senescence Assay Kit (SA-β-gal Staining)
2. AKR-100: β Galactosidase Staining Kit

Kit Components

1. 2X Cell Lysis Buffer (Part No. 123101): One bottle – 10 mL
2. 2X Reaction Buffer (Part No. 123102): One bottle – 10 mL
3. SA-ß-Gal Substrate (20X) (Part No. 123103): One amber tube – 300 µL
4. Stop Solution (Part No. 123104): One bottle – 25 mL

Materials Not Supplied

1. Senescence cells or tissue samples
2. 37^0 C 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.

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.

Reagents	96-well	24-well	6-well	10 cm
1X Cell Lysis Buffer	50 µL	200 µL	500 µL	750 µL
2X Assay Buffer	50 µL	200 µL	500 µL	750 µL

Assay Protocol

1. Aspirate the medium from the senescence cells.
2. Wash the cells once with 200 µL of cold 1X PBS and aspirate the wash.
3. Add 50 µL of cold 1X Cell Lysis Buffer. Incubate at 4 ^{0}C for 5 minutes.
4. (optional) Determine the total protein concentration of each sample by protein assay such as Pierce's BCA protein Assay.
5. Add 50 µL of freshly prepared 2X Assay Buffer. Incubate the wells at 37 ^{0}C 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 365 nm (Excitation)/502 nm (Emission).

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.

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

©2007-2008: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.