

---

Product Manual

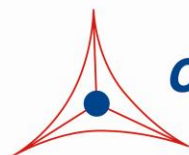
# 96-Well Cellular Senescence Assay Kit (SA- $\beta$ -gal Activity, Fluorometric Format)

Catalog Number

CBA-231	120 assays
CBA-231-5	5 x 120 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<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 kit provides sufficient quantities to perform up to 120 assays in a 96-well plate.

## **Related Products**

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

## **Kit Components (shipped at room temperature)**

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

## **Materials Not Supplied**

1. Senescent cells or tissue samples
2.  $\beta$ -mercaptoethanol
3. 96-well plate suitable for a fluorescence plate reader
4. 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.

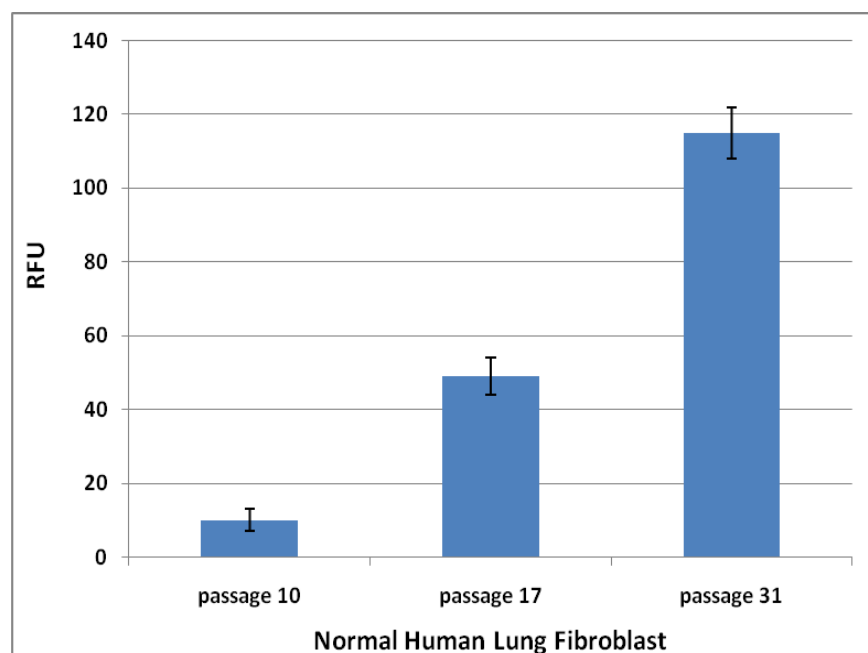
Reagent	96-well	24-well	6-well	10 cm
1X Cell Lysis Buffer	100 $\mu$ L	400 $\mu$ L	1000 $\mu$ L	1500 $\mu$ L

## **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. 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.

## 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. Shen, C.Y. et al. (2023). Unveiling the molecular basis of inflamm-aging induced by advanced glycation end products (AGEs)-modified human serum albumin (AGE-HSA) in patients with different immune-mediated diseases. *Clin Immunol.* **252**:109655. doi: 10.1016/j.clim.2023.109655.
2. Ambrosio, M.R. et al. (2023). Targeting G-quadruplex motifs interferes with differentiation of adipose-derived mesenchymal stem cells. *Stem Cell Res Ther.* **14**(1):98. doi: 10.1186/s13287-023-03320-9.
3. Choi, D. et al. (2023). Vertical Vibration of Mouse Osteoblasts Promotes Cellular Differentiation and Cell Cycle Progression and Induces Aging In Vitro. *Biomedicines.* **11**(2):444. doi: 10.3390/biomedicines11020444.
4. Nelczyk, A.T. et al. (2022). The nuclear receptor TLX (NR2E1) inhibits growth and progression of triple- negative breast cancer. *Biochim Biophys Acta Mol Basis Dis.* **1868**(11):166515. doi: 10.1016/j.bbadis.2022.166515.
5. Chelyapov, N. et al. (2022). Autologous NK cells propagated and activated ex vivo decrease senescence markers in human PBMCs. *Biochem Biophys Rep.* doi: 10.1016/j.bbrep.2022.101380.
6. Furukawa, M. et al. (2022). Analysis of senescence in gingival tissues and gingival fibroblast cultures. *Clin Exp Dent Res.* doi: 10.1002/cre2.581.

7. Kelleher, A.M. et al. (2021). Deficiency of PARP-1 and PARP-2 in the mouse uterus results in decidualization failure and pregnancy loss. *Proc Natl Acad Sci U S A*. **118**(40):e2109252118. doi: 10.1073/pnas.2109252118.
8. Deng, Z. et al. (2021). Biofunction of Polydopamine Coating in Stem Cell Culture. *ACS Appl Mater Interfaces*. doi: 10.1021/acsami.0c22565.
9. Lee, Y.N. et al. (2021). Ultrasonic microbubble VEGF gene delivery improves angiogenesis of senescent endothelial progenitor cells. *Sci Rep*. **11**(1):13449. doi: 10.1038/s41598-021-92754-3.
10. Hirata, Y. et al. (2021). Advanced maternal age induces fetal growth restriction through decreased placental inflammatory cytokine expression and immune cell accumulation in mice. *J Reprod Dev*. doi: 10.1262/jrd.2021-034.
11. Mehdi, S.J. et al. (2021). Normal and cancer fibroblasts differentially regulate TWIST1, TOX and cytokine gene expression in cutaneous T-cell lymphoma. *BMC Cancer*. **21**(1):492. doi: 10.1186/s12885-021-08142-7.
12. Bourdon, B. et al. (2021). Marine Collagen Hydrolysates Promote Collagen Synthesis, Viability and Proliferation While Downregulating the Synthesis of Pro-Catabolic Markers in Human Articular Chondrocytes. *Int. J. Mol. Sci.* **22**(7):3693. doi: 10.3390/ijms22073693.
13. Kong, C.S. et al. (2021). Embryo biosensing by uterine natural killer cells determines endometrial fate decisions at implantation. *FASEB J*. **35**(4):e21336. doi: 10.1096/fj.202002217R.
14. Baxley, R.M. et al. (2021). Bi-allelic MCM10 variants associated with immune dysfunction and cardiomyopathy cause telomere shortening. *Nat Commun*. **12**(1):1626. doi: 10.1038/s41467-021-21878-x.
15. Yamaguchi, S. et al. (2021). Characterization of an active LINE-1 in the naked mole-rat genome. *Sci Rep*. **11**(1):5725. doi: 10.1038/s41598-021-84962-8.
16. Bourdon, B. et al. (2021). Marine Collagen Hydrolysates Downregulate the Synthesis of Pro-Catabolic and Pro-Inflammatory Markers of Osteoarthritis and Favor Collagen Production and Metabolic Activity in Equine Articular Chondrocyte Organoids. *Int J Mol Sci*. **22**(2):E580. doi: 10.3390/ijms22020580.
17. Jiang, Y. et al. (2021). Histone H3K27 methyltransferase EZH2 and demethylase JMJD3 regulate hepatic stellate cells activation and liver fibrosis. *Theranostics*. **11**(1):361-378. doi: 10.7150/thno.46360.
18. Mogilenko, D.A. et al. (2020). Comprehensive Profiling of an Aging Immune System Reveals Clonal GZMK<sup>+</sup> CD8<sup>+</sup> T Cells as Conserved Hallmark of Inflammaging. *Immunity*. doi: 10.1016/j.immuni.2020.11.005.
19. Setoguchi, Y. et al. (2020). Functional assessment of retinal pigment epithelium cell transplants with various degrees of pigmentation for age-related macular degeneration. *Kawasaki Medical Journal*. **46**:49-58. doi: 10.11482/KMJ-E202046049.
20. Rostami, A. et al. (2020). Senescence, Necrosis, and Apoptosis Govern Circulating Cell-free DNA Release Kinetics. *Cell Rep*. **31**(13):107830. doi: 10.1016/j.celrep.2020.107830.
21. Pacifici, F. et al. (2020). Prdx6 Plays a Main Role in the Crosstalk Between Aging and Metabolic Sarcopenia. *Antioxidants (Basel)*. **9**(4). pii: E329. doi: 10.3390/antiox9040329.
22. Lin, X. et al. (2020). Excessive oxidative stress in cumulus granulosa cells induced cell senescence contributes to endometriosis-associated infertility. *Redox Biol*. **30**:101431. doi: 10.1016/j.redox.2020.101431.
23. Ohigashi, T. et al. (2019). Protective effect of phosphatidylcholine on lysophosphatidylcholine-induced cellular senescence in cholangiocyte. *J Hepatobiliary Pancreat Sci*. doi: 10.1002/jhbp.684.

24. Takagi, H. et al. (2019). Blockade of  $\gamma$ -Glutamylcyclotransferase Enhances Docetaxel Growth Inhibition of Prostate Cancer Cells. *Anticancer Res.* **39**(9):4811-4816. doi: 10.21873/anticancer.13666.
25. Tencerova, M. et al. (2019). Obesity-Associated Hypermetabolism and Accelerated Senescence of Bone Marrow Stromal Stem Cells Suggest a Potential Mechanism for Bone Fragility. *Cell Rep.* **27**(7):2050-2062.e6. doi: 10.1016/j.celrep.2019.04.066.
26. Morsczech, C. et al. (2019). Short telomeres correlate with a strong induction of cellular senescence in human dental follicle cells. *BMC Mol Cell Biol.* **20**(1):5. doi: 10.1186/s12860-019-0185-4.
27. Cho, S.Y. et al. (2019). Oxytocin Alleviates Cellular Senescence through Oxytocin Receptor-Mediated ERK/Nrf2 Signalling. *Br J Dermatol.* doi: 10.1111/bjd.17824.
28. Cao, J. et al. (2019). Combining CDK4/6 inhibition with taxanes enhances anti-tumor efficacy by sustained impairment of pRB-E2F pathways in squamous cell lung cancer. *Oncogene.* doi: 10.1038/s41388-019-0708-7.
29. Mehdi, S.J. et al. (2019). Mesenchymal stem cells gene signature in high-risk myeloma bone marrow linked to suppression of distinct IGFBP2-expressing small adipocytes. *Br J Haematol.* **184**(4):578-593. doi: 10.1111/bjh.15669.
30. Velusami, C.C. et al. (2018). Polar extract of Curcuma longa protects cartilage homeostasis: possible mechanism of action. *Inflammopharmacology.* **26**(5):1233-1243. doi: 10.1007/s10787-017-0433-1.

## **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.  
 5628 Copley Drive  
 San Diego, CA 92111  
 Worldwide: +1 858-271-6500  
 USA Toll-Free: 1-888-CBL-0505  
 E-mail: [tech@cellbiolabs.com](mailto:tech@cellbiolabs.com)  
[www.cellbiolabs.com](http://www.cellbiolabs.com)

©2007-2024: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.