Product Manual

Cellular Senescence Assay Kit (SA-β-gal Staining)

Catalog Number

CBA-230	50 assays
CBA-230-5	5 x 50 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 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 Cellular Senescence Assay Kit provides an easy-to-use and efficient method to determine cellular senescence. SA-β-galactosidase catalyzes the hydrolysis of X-gal, which produces a blue color. Each kit provides sufficient quantities to perform up to 50 assays in 35 mm dishes.

Related Products

1. CBA-231: 96-Well Cellular Senescence Assay Kit (SA-β-gal Activity, Fluorometric Format)
2. CBA-232: Quantitative Cellular Senescence Assay (SA β-Gal)
3. CBA-240: CytoSelect™ Cell Viability and Cytotoxicity Assay
4. AKR-100: β Galactosidase Staining Kit

Kit Components

1. 100X Fixing Solution (Part No. 40010): One tube – 1.5 mL of 25% Glutaraldehyde
2. Staining Solution A (Part No. 40011): One tube – 1.5 mL of 500 mM Potassium Ferrocyanide
3. Staining Solution B (Part No. 40012): One tube – 1.5 mL of 500 mM Potassium Ferricyanide
4. Staining Solution C (Part No. 40015): One bottle – 4.5 mL of 1 M Citrate-Na_2HPO_4 Buffer, pH 6.0, 50 mM $MgCl_2$
5. Staining Solution D (Part No. 40016): One bottle – 4.0 mL of 5 M NaCl
6. X-gal Solution (Part No. 40014): Two tubes – 1.5 mL of 40 mg/mL X-gal in DMF in each tube

Materials Not Supplied

1. PBS
2. Light microscope
3. Senescent cells or tissue samples

Storage

Store X-gal solution protected from light at -20ºC. Store all other components at 4ºC.

Preparation of Reagents

- 1X Fixing Solution: Prepare a 1X Fixing Solution by diluting the provided 100X stock 1:100 in 1X PBS. Store the diluted solution at room temperature for up to six months.
- Cell Staining Working Solution: Prepare FRESH cell staining working solution based on the number of samples. The chart below is suggested for samples in 35 mm plate, and may be modified accordingly to suit other culture plate sizes.

Reagents	1 dish (35 mm)	5 dishes (35 mm)	10 dishes (35 mm)
Staining Solution A	20 µL	100 µL	200 µL
Staining Solution B	20 µL	100 µL	200 µL
Staining Solution C	80 µL	400 µL	800 µL
Staining Solution D	60 µL	300 µL	600 µL
X-Gal Solution	50 µL	250 µL	500 µL
H_2O	1.77 mL	8.85 mL	17.7 mL
Total	**2 mL**	**10 mL**	**20 mL**

Assay Protocol (35 mm dish)

1. Aspirate the medium from the senescent cells expressing SA-ß-Gal.
2. Wash the cells twice with 3 mL of 1X PBS and aspirate the final wash.
3. Add 2 mL of 1X Fixing Solution. Incubate at room temperature for 5 minutes.
4. Remove the fixing solution and wash the fixed cells three times with 3 mL of 1X PBS.
5. Aspirate the final wash, and completely cover cells by adding 2 mL of freshly prepared Cell Staining Working Solution.
6. Incubate the cells at 37ºC protected from light for 4 hr to overnight.
7. Remove the Cell Staining Working Solution, then wash the stained cells twice with 3 mL of 1X PBS and store cells in 1X PBS. For long-term storage, overlay the cells with 1X PBS containing 20% Glycerol. Store at 4ºC.

Note: Excess amount of salt crystals can be removed by briefly incubating the stained sample with DMSO.

8. Count the blue stained senescence cells using light microscope.

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. Ryu, S. et al. (2023). Impact of media compositions and culture systems on the immunophenotypes of patient-derived breast cancer cells. *BMC Cancer*. **23**(1):831. doi: 10.1186/s12885-023-11185-7.
2. Raber, J. et al. (2023). Behavioral and Cognitive Performance Following Exposure to Second-Hand Smoke (SHS) from Tobacco Products Associated with Oxidative-Stress-Induced DNA Damage and Repair and Disruption of the Gut Microbiome. *Genes*. **14**(9):1702. doi: 10.3390/genes14091702.
3. Warman, D.J. et al. (2023). Effects of Thyme (Thymus vulgaris L.) Essential Oil on Aging-Induced Brain Inflammation and Blood Telomere Attrition in Chronologically Aged C57BL/6J Mice. *Antioxidants*. **12**(6):1178. doi: 10.3390/antiox12061178.
4. Ji, M.L. et al. (2023). Dynamic chromatin accessibility tuning by the long noncoding RNA ELDR accelerates chondrocyte senescence and osteoarthritis. *Am J Hum Genet*. **110**(4):606-624. doi: 10.1016/j.ajhg.2023.02.011.
5. Huang, Y. et al. (2022). The impact of senescence on muscle wasting in chronic kidney disease. *J Cachexia Sarcopenia Muscle*. doi: 10.1002/jcsm.13112.
6. Sanagawa, A. et al. (2022). Effect of Replicative Senescence on the Expression and Function of Transporters in Human Proximal Renal Tubular Epithelial Cells. *Biol Pharm Bull*. **45**(11):1636-1643. doi: 10.1248/bpb.b22-00322.
7. Okawa, R. et al. (2022). The effects of continuous exposure to low-dose chlorine dioxide gas on the characteristics of induced pluripotent stem cells. *Regen Ther*. **21**:250-257. doi: 10.1016/j.reth.2022.07.014.
8. Yamamoto, M. et al. (2022). Gemcitabine Cooperates with Everolimus to Inhibit the Growth of and Sensitize Malignant Meningioma Cells to Apoptosis Induced by Navitoclax, an Inhibitor of Anti-Apoptotic BCL-2 Family Proteins. *Cancers (Basel)*. **14**(7):1706. doi: 10.3390/cancers14071706.
9. Madonna, R. et al. (2022). Sex-related differential susceptibility to ponatinib cardiotoxicity and differential modulation of the Notch1 signalling pathway in a murine model. *J Cell Mol Med*. doi: 10.1111/jcmm.17008.
10. Das, J.K. et al. (2022). Elongation factor-2 kinase is a critical determinant of the fate and antitumor immunity of CD8+ T cells. *Sci Adv*. **8**(5):eabl9783. doi: 10.1126/sciadv.abl9783.
11. Wang, T. et al. (2021). Pulsed electromagnetic fields attenuate glucocorticoid-induced bone loss by targeting senescent LepR+ bone marrow mesenchymal stromal cells. *Mater Sci Eng C Mater Biol Appl*. doi: 10.1016/j.msec.2021.112635.
12. Madonna, R. et al. (2021). Sodium-glucose cotransporter type 2 inhibitors prevent ponatinib-induced endothelial senescence and disfunction: A potential rescue strategy. *Vascul Pharmacol*. **142**:106949. doi: 10.1016/j.vph.2021.106949.
13. Wang, Q. et al. (2021). Celecoxib prevents tumor necrosis factor-α (TNF-α)-induced cellular senescence in human chondrocytes. *Bioengineered*. **12**(2):12812-12820. doi: 10.1080/21655979.2021.2003661.
14. Kim, N.Y. et al. (2021). Temozolomide abrogates the aggressiveness of urothelial carcinoma cells by enhancing senescence and depleting the side population. *Oncol Lett*. **22**(6):845. doi: 10.3892/ol.2021.13106.

15. Jenuit, M. et al (2021). Establishment and Cryopreservation of Fibroblast Cell Line from a Sumatran Rhinoceros (Dicerorhinus sumatrensis). *J. Sustain. Sci. Manag*. **16**(4):85-98. doi: 10.46754/jssm.2021.06.008.
16. Ho, D.H. et al. (2021). LRRK2 Kinase Inhibitor Rejuvenates Oxidative Stress-Induced Cellular Senescence in Neuronal Cells. *Oxid Med Cell Longev*. doi: 10.1155/2021/9969842.
17. Cui, Z. et al. (2021). Effect of the traditional Chinese medicine Pinggan-Qianyang decoction on SIRT1-PTEN signaling in vascular aging in spontaneously hypertensive rats. *Hypertens Res*. doi: 10.1038/s41440-021-00682-6.
18. Zhang, Y. et al. (2021). Salidroside Ameliorates Vascular Endothelial Cell Senescence through Downregulation of KLF4. *J Biosci Med (Irvine)*. **9**(2):21-32. doi: 10.4236/jbm.2021.92003.
19. Kim, S.N. et al. (2020). Culturing at Low Cell Density Delays Cellular Senescence of Human Bone Marrow-Derived Mesenchymal Stem Cells in Long-Term Cultures. *Int J Stem Cells*. doi: 10.15283/ijsc20078.
20. Baek, A.R. et al. (2020). Spermidine attenuates bleomycin-induced lung fibrosis by inducing autophagy and inhibiting endoplasmic reticulum stress (ERS)-induced cell death in mice. *Exp Mol Med*. doi: 10.1038/s12276-020-00545-z.
21. Hwang, S.G. et al. (2020). Cold atmospheric plasma prevents wrinkle formation via an anti-aging process. *Plasma Med*. doi: 10.1615/PlasmaMed.2020034810.
22. Madonna, R. et al. (2020). Empagliflozin reduces the senescence of cardiac stromal cells and improves cardiac function in a murine model of diabetes. *J Cell Mol Med*. doi: 10.1111/jcmm.15699.
23. Takei, Y. et al. (2020). Quality assessment tests for tumorigenicity of human iPS cell-derived cartilage. *Sci Rep*. **10**(1):12794. doi: 10.1038/s41598-020-69641-4.
24. Azmi, S.M. et al. (2020). Human umbilical cord-mesenchymal stem cells: a promising strategy for corneal epithelial regeneration. *Regen Med*. doi: 10.2217/rme-2019-0103.
25. Yamazaki, H. et al. (2020). Ribosome binding protein GCN1 regulates the cell cycle and cell proliferation and is essential for the embryonic development of mice. *PLoS Genet*. **16**(4):e1008693. doi: 10.1371/journal.pgen.1008693.
26. Sugimoto, H. et al. (2020). Primary culture of mouse adipose and fibrous synovial fibroblasts under normoxic and hypoxic conditions. *Biomed Res*. **41**(1):43-51. doi: 10.2220/biomedres.41.43.
27. Sogawa, K. et al. (2020). Effects of continuous exposure to low concentration of ClO2 gas on the growth, viability, and maintenance of undifferentiated MSCs in long-term cultures. *Regen Ther*. **14**:184-190. doi: 10.1016/j.reth.2019.12.007.
28. Tan, J. et al. (2019). An R-loop-initiated CSB-RAD52-POLD3 pathway suppresses ROS-induced telomeric DNA breaks. *Nucleic Acids Res*. pii: gkz1114. doi: 10.1093/nar/gkz1114.
29. O'Hara, S.P. et al. (2019). The transcription factor ETS1 promotes apoptosis resistance of senescent cholangiocytes by epigenetically up-regulating the apoptosis suppressor BCL2L1. *J Biol Chem*. pii: jbc.RA119.010176. doi: 10.1074/jbc.RA119.010176.
30. Cilibrasi, C. et al. (2019). A Ploidy Increase Promotes Sensitivity of Glioma Stem Cells to Aurora Kinases Inhibition. *Journal of Oncology*. doi: 10.1155/2019/9014045.

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