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

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 wells.

Related Products

1. CBA-080: CytoSelect™ 24-Well Anoikis Assay
2. CBA-081: CytoSelect™ 96-Well Anoikis Assay
3. CBA-231: 96-Well Cellular Senescence Assay Kit (SA-β-gal Activity, Fluorometric Format)
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. 100X Fixing Solution (Part No. 90010): One tube – 1.5 mL of 25% Glutaraldehyde
2. Staining Solution A (Part No. 90011): One tube – 1.5 mL of 500 mM Potassium Ferrocyanide
3. Staining Solution B (Part No. 90012): One tube – 1.5 mL of 500 mM Potassium Ferricyanide
4. Staining Solution C (Part No. 90015): Three tubes – 1.5 mL of 1 M Citrate-Na_2HPO_4 Buffer, pH 6.0, 50 mM $MgCl_2$ in each tube
5. Staining Solution D (Part No. 90016): Two tubes – 2.0 mL of 5 M NaCl in each tube
6. X-gal Solution (Part No. 90014): Two tubes – 1.5 mL of 40 mg/mL X-gal in DMF in each tube

Materials Not Supplied

1. PBS
2. 37ºC Incubator
3. Light microscope
4. 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 4^0 C.

   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.

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