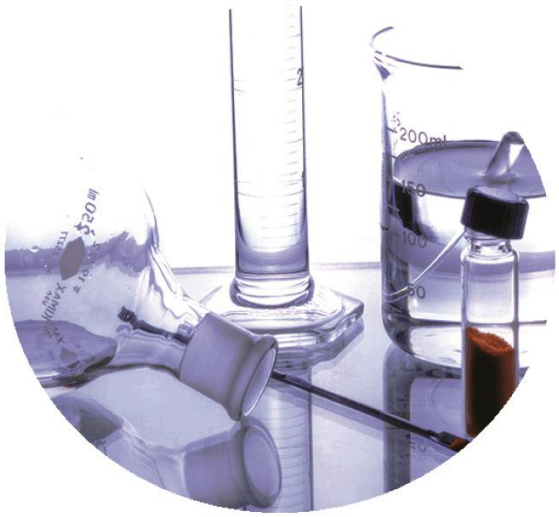

Assay kits

Cellular Senescence Kit

Cellular Senescence kit

Protocol

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IMPORTANT NOTES – Before you begin

Ready-to-use assay system for determining the cellular senescence based on senescence-associated β-galactosidase (SA X-gal) staining

- ✓ After a long period of normal growth, cells in culture showed abnormalities typical of the Hayflick model. The cells vary in size and shape, the cytoplasm begins to be granular with many cell inclusions and debris is formed in the medium. Senescence goes with decreasing of proliferation potential, telomere shortening and impairment of cell function (*i.e* decrease of differentiation potential of stem cells).
- ✓ During senescence in mammalian cells, an endogenous lysosomal β-galactosidase is over-expressed and is accumulated within the aging cells.
- ✓ The presence of this SA X-gal activity allows visualization of aging cell population *in vitro*.

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

www.ozbiosciences.com

Any questions?

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Cellular Senescence Kit | Specifications

Package content	GXS0003 Fixing Buffer (125 ml) 10X PBS (75 ml) Staining Buffer (125 ml) 25X Stock Solution of SA X-Gal (4 X 1 ml) The kit contains sufficient reagents to perform 50 assays of 60 mm dishes.
Shipping conditions	The kit is shipped with gel pack (4°C).
Storage conditions	Upon receipt and for long-term use, store all reagent tubes at the indicated storage conditions: Fixing Buffer (+4°C) 10X PBS (+4°C) Staining Buffer (+4°C) 25X Stock Solution of SA X-Gal (-20°C)
Shelf life	1 year from the date of purchase when properly stored and handled
Important notice	For research use only. Not for use in diagnostic procedures.

1. Usage

1. Fix the cells with the fixing buffer
2. Stain the cells with SA X-gal staining solution
3. Observe the cells with blue stain under a microscope
4. Calculate the percentage of blue cells in the total population.

2. General Considerations

- Before use, dilute the 10X PBS to 1X with deionized water. The surplus of unused 1X PBS may be stored at +4°C or room temperature for future use.

- Dilute the 25X stock solution of SA X-Gal to 1X with Staining Buffer. The 1X solution of SA X-Gal must be prepared freshly each time the assay is performed. Discard the surplus of SA X-Gal solution.

- IMPORTANT
  The signal intensity can be cell type dependent. Accordingly, for some cells, such as fibroblasts or epithelial cells and if the blue signal is too weak, we recommend to lower the pH of the Staining solution from 7.4 to 6-6.5.

3. General Protocol

1. Wash the cells once with 1X PBS.

2. Add Fixing Buffer to the dish and incubate for 10-15 minutes at room temperature.
   CAUTION: The Fixing Buffer contains chemicals that are corrosive, carcinogenic and poisonous and must be handled carefully (see Materials Safety Data Sheet for further details). Wear gloves, goggles, lab coats and other protective gear when handling the Fixing Buffer. Some products are harmful if inhaled, swallowed or absorbed through the skin.

3. Remove the fixing solution from the culture dish and carefully wash the cells twice with 1X PBS.

4. Add the freshly prepared 1X staining solution of SA X-Gal to the dish. Incubate the cells between 1 to 18 hours at +37°C in a humidified incubator.

NOTE: *The signal intensity can be cell type dependent. Accordingly, for some cells, such as fibroblasts or epithelial cells and if the blue signal is too weak, we recommend to lower the pH of the Staining solution from 7.4 to 6-6.5.*

5. Remove the SA X-Gal staining solution and wash the cells once with 1X PBS.

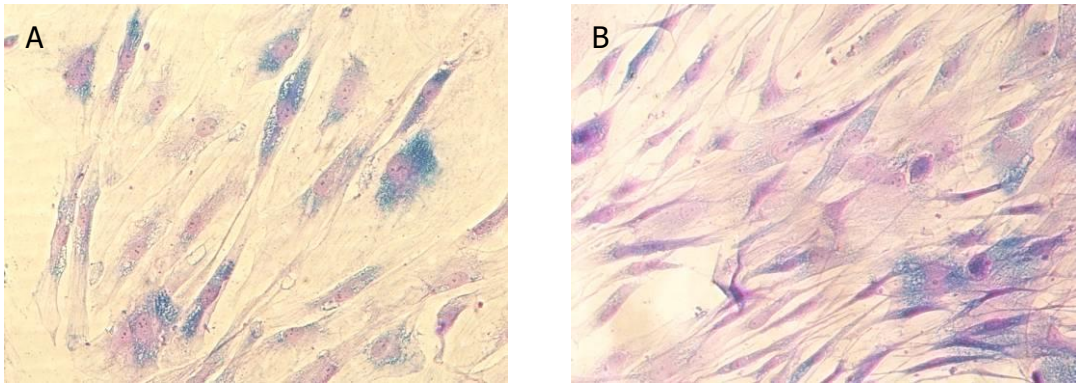
6. Add 1X PBS to the dish and proceed to the examination of the blue cells under a light microscope; count the stained and unstained cells in randomly selected fields. Calculate the percentage of stained cells in the total population.

8. If you need to store the plates for several weeks or months, fix each well with 1ml of 10% formalin in PBS (not supplied) for 10 minutes at room temperature, rinse with 1X PBS and store in 1X PBS or 70% glycerol solution (not supplied) at +4°C

Volumes of solution recommended for various culture dishes are listed in the table below.

Type of culture dish	Fixing Buffer (µl/well)	Staining Buffer (µl/well)	1X PBS Washing Buffer (µl/well/wash)
Chambered slide	500	500	1000
24-well plate	250	250	500
12-well plate	500	500	1000
6-well plate	1000	1000	2000
60 mm dish	2500	2500	3000
100 mm dish	5000	5000	8000

Example of staining obtained on senescent stem cells



Adipose derived Stem Cells at passage 7 (A) and Mesenchymal Stem Cells at passage 6 (B) stained with the Cellular Senescence Kit (nuclei counterstained with Crystal Violet solution, not supplied).

NOTES

Additional products

- **OZBlue Cell Viability kit** for cell viability measurement
- **ROS Detection Assay Kit** for measuring Reactive Oxygen Species activity within your cells

Purchaser Notification

Limited License
The purchase of the Cellular Senescence Kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). This reagent is intended for in-house research only by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the Cellular Senescence Kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all Cellular Senescence Kit reagents and documentation to OZ Biosciences, or by destroying all D-Luciferin components. Purchasers are advised to contact OZ Biosciences with the notification that a Cellular Senescence Kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s). This document covers entirely the terms of the Cellular Senescence Kit research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations
Cellular Senescence Kit and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

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