Cellular Senescence Kit

INSTRUCTION MANUAL



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Instruction Manual

1.1. Description

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

Catalog Number: GXS0003

You can order this product by contacting us. For all other additional information, do not hesitate to contact our dedicated technical support (tech@ozbiosciences.com).

1. Technology

1.1. Description

Congratulations on your purchase of the Cellular Senescence Kit!

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*.

This Cellular Senescence Kit is:

- Simple and Rapid
- Ready-to-use
- Economical

1.2. Kit Contents

The kit contains sufficient reagents to perform 50 assays of 60 mm dishes.

Component	Quantity	Storage
Fixing Buffer	125 ml	4°C
10X PBS	75 ml	4°C
Staining Buffer	125 ml	4°C
25X Stock Solution of SA X-Gal	4 X 1 ml	-20°C

Stability and Storage

Storage — Upon receipt and for long-term use, store all reagent tubes at the indicated storage conditions (see table above). Kit's components are stable for at least 1 year at the recommended storage temperature.

Shipping condition — The SA X-Gal Staining kit is shipped with gel pack (4°C)

2. Applications and Protocols

2.1. 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.

2.2. 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.

2.3. 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.

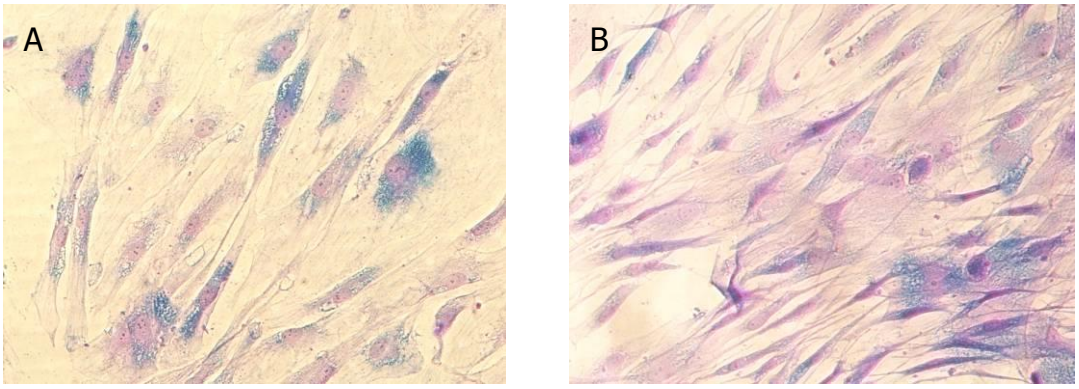
6. Add 1X PBS to the dish and proceed to the examination of the blue cells under a light microscope; count the stained and unstained cells in randomly selected fields. Calculate the percentage of stained cells in the total population.

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).

3. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate *(standard size for all cell culture support)* Mega Magnetic plate *(mega size to hold 4 culture dishes at one time)*
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
Magnetofectamine™ (*for all nucleic acids*)
NeuroMag *(dedicated for neurons)*
SilenceMag *(for siRNA application)*
Viral Transduction enhancers:
ViroMag (*to optimize viral transduction*) / AdenoMag *(for Adeno viruses)*
ViroMag R/L *(specific for retrovirus and Lentivirus)*
AdenoMag *(for adenovirus and aav)*
***In vivo* Targeted Delivery**
In vivo PolyMag (*for all nucleic acids*)
In vivo DogtorMag (*for all nucleic acids*)
In vivo ViroMag *(for all viral vectors)*
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
DreamFect Stem *(Transfection reagent for pluripotent stem cells)*
VeroFect *(for Vero cells)*
FlyFectin *(for Insect cells)*
I-MICST TECHNOLOGY
Viro-MICST (*to transduce directly on magnetic cell purification columns)*
Capture and Concentration of Virus
Mag4C *(to capture, concentrate and conserve viruses)*
3D TRANSFECTION TECHNOLOGY
3Dfect *(for scaffolds culture)*
3DfectIN *(for hydrogels culture)*
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (*for **H**igh **Y**ield **P**rotein **E**xpression)*
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN *(delivery reagent for antibodies)*
Pro-DeliverIN *(delivery reagent for protein in vivo and in vitro)*
PLASMIDS PVECTOZ
pVectOZ-LacZ
pVectOZ-SEAP
ASSAY KITS
Bradford – Protein Assay Kit
MTT cell proliferation kit
β-Galactosidase assay kits (CPRG/ONPG)
BIOCHEMICALS
D-Luciferin, K^+ and Na^+
X-Gal powder 1g / G-418, Sulfate

Do not hesitate to contact us for all complementary information and remember to visit our website in order to stay inform on our last breakthrough technologies and updated on our complete product list. http://www.ozbiosciences.com

NOTES

Purchaser Notification

Limited License

The purchase price paid for the Cellular Senescence kit by end users grants them a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in the section 1, Kit Contents). This reagent is intended **for internal research only** by the buyer. Such use is limited to the use in the product manual. Furthermore, 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.

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Director of Business Development
OZ Biosciences SAS
Parc Scientifique et Technologique de Luminy
Bâtiment Grand Luminy technopole
Case 922 zone entreprise
13288 Marseille Cedex 9, France
Ph: +33 (0)4.86.94.85.16
Fax: +33 (0)4.86.94.85.15
E-mail: contact@ozbiosciences.com

EUROPE & ASIA
OZ Biosciences SAS
163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
France
Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515
contact@ozbiosciences.com
order@ozbiosciences.com
tech@ozbiosciences.com

USA & CANADA
OZ Biosciences INC
4901 Morena Blvd
Suite 901
San Diego CA 92117
USA
Ph: + 1-858-246-7840
Fax: + 1-855-631-0626
contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com
techUSA@ozbiosciences.com

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