PRODUCT DATASHEET
SenTraGor™ Reagent - Antibody-enhanced detection of Senescent cells

Cat no: AR8850020, AR8850040, AR8850080

Overview

Product Name

SenTraGor™ – Antibody-enhanced detection of senescent cells

Description

A biotin linked Sudan Black B (SBB) analogue tracing lipofuscin in senescent cells.

Background

Cellular senescence is a biological process involved in normal embryonic and adult life and increases with age. It also occurs in the frame of various diseases and after therapeutic interventions. Detection and measurement of senescent cells is critical and highly desired in research and clinical practice. Senescent cells are found in a wide spectrum of age related disorders, including cancer. SenTraGor™, an SBB analogue conjugated with biotin, reacts with lipofuscin granules that have been shown to accumulate during the senescence process.

References

1. Evangelou K., Lougiakis N., Rizou S.V., et al. 2017. Robust, universal biomarker assay to detect senescent cells in biological specimens. Aging Cell 16, pp 192-197.
2. Georgakopoulou E.A., Tsimaratou K., Evangelou K., et al. 2013. Specific lipofuscin staining as a novel biomarker to detect replicative and stress-induced senescence. A method applicable in cryo-preserved and archival tissues. Aging (Albany NY). 5, pp 37-50.
3. Campisi J., d'Adda di Fagagna F 2007. Cellular senescence: when bad things happen to good cells. Nat. Rev. Mol. Cell Biol 8, pp 729-740.
4. Gorgoulis V.G., Halazoneitis T.D. 2010. Oncogene- induced senescence: the bright and dark side of the response. Curr Opin Cell Biol 22, pp 816-827.
5. Bujarrabal A., Schumacher B. 2017. Tracking senescent cells: A new biomarker assay opens new avenues in senescence research. Mech Ageing Dev. 162, pp 106-107.
6. Salmonowicz H., Passos J.F. 2017. Detecting senescence: a new method for an old pigment. Aging Cell. 16, pp 432-434.
7. Childs B.G., Gluscevic M., Baker D.J., et al. 2017. Senescent cells: an emerging target for diseases of ageing. Nat Rev Drug Discov. Jul 21.

Source

SenTraGor™ is a chemical synthetic biotinylated compound, analogue of SBB stain. Its synonym is (2-Methyl-6-((E)-(4-((E)-phenyldiazenyl) naphthalen- 1-yl)diazenyl)-2,3-dihydro-1H-perimidin-2-yl) methyl 5-((3aR,4R,6aS)-2-oxohexahydro-1H-thieno[3,4-d] imidazol-4-yl) pentanoate.

Product description

Each vial contains 20 mg (Cat no: AR8850020), 40 mg (Cat no: AR8850040) or 80 mg (Cat no: AR8850080) of solid SenTraGor™ compound. To dissolve the compound, add 3.5-3.75 ml or 7-7.5 ml or 14-15 ml, respectively, of 100% Ethanol and cover with parafilm.

The ideal concentration depends on the examined biological material and its processing and can be determined as follows: start with 3.5ml or 7 ml or 14 ml volume of 100% Ethanol. If non-specific ("background") reaction of the reagent is observed adjust volume to 3.75ml or 7.5 ml or 15 ml, respectively. Incubate at 56°C in a water bath for 120 min until the compound is completely dissolved. Before use, filter with a syringe using 13 mm filter and 0.22 μm membrane.

Storage

Store at room temperature. Protect from light. After reconstitution store at room temperature, protect from light and air tight seal. The reagent is stable for up to 2 months after reconstitution at room temperature. Alternatively, you can aliquot the reconstituted reagent, air tight seal, protected from light and store at room temperature for up to 8 months.

Applications

SenTraGor™ is recommended for the recognition of senescent cells. It binds to cytoplasmic lipofuscin, a hallmark of senescent cells. It can be applied on cells from aspiration or cell cultures as well as on tissue sections from frozen or formalin-fixed paraffin embedded (FFPE) samples. It can be used for immunohistochemistry (IHC), immunocytochemistry (ICC), immunofluorescence (IF) and flow cytometry analysis (FACS). Application of hybrid histochemical/ immunohistochemical assay is required to produce a consistent and specific signal. Use a primary anti-biotin antibody and secondary antibody against your primary anti-biotin antibody.
For more details refer to step by step protocols at www.sentragortech.com

Controls

a.Positive controls:

Cellular systems that can be used as positive controls are cells undergoing replicative senescence (RS), for example primary human diploid lung fibroblasts (DLFs) at late passages of division or cells exhibiting Stress induced senescence (SISP), for example irradiated primary human DLFs, or inducible Saos2 p21WAF1/CIP1 Tet-ON cells, when p21WAF1/CIP1 is activated. Tissues from animal models and human clinical cases with established senescence can serve as positive controls (examples: K-RAS induced mouse lung adenomas, irradiated human laryngeal lesions).

b.Negative controls:

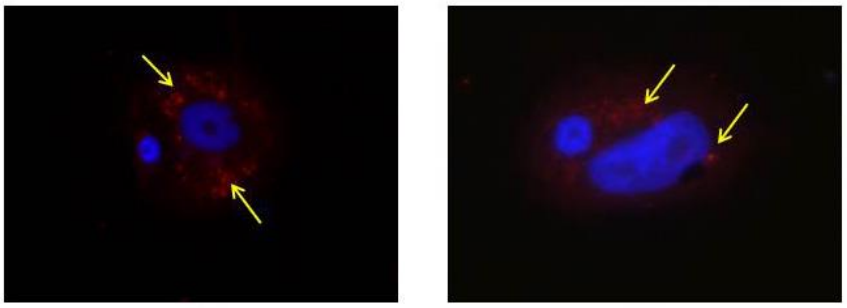
Cellular systems that can be used as negative controls are early proliferative passages of primary human DLFs, Saos2 p21WAF1/CIP1 Tet-ON cell when p21WAF1/CIP1 is OFF (not induced). Normal, non-aged, tissues can be used as negative controls.

c.References:

1) Evangelou K., Lougiakis N., Rizou S.V., et al. 2017. Robust, universal biomarker assay to detect senescent cells in biological specimens. Aging Cell 16, pp 192-197; 2) Galanos P., Vougas K., Walter D., et al. 2016. Chronic p53-independent p21 expression causes genomic instability by deregulating replication licensing. Nat Cell Biol. 18, pp 777-89; 3) Collado M., Gil J., Efeyan A., et al. 2005. Tumour biology: senescence in premalignant tumours. Nature. 436, pp 42.

Presented Data

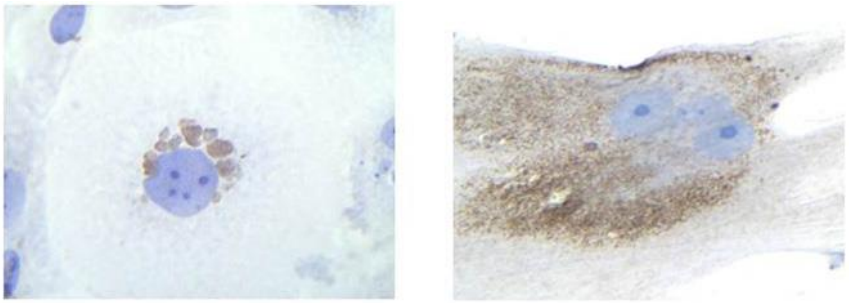
Hybrid cytochemistry/Immunofluorescence



(A) (B)

Arrows indicate positive SenTraGor™ staining (Texas Red) in senescent U2OS Cdt1 Tet-ON cells (A,B). Counterstain: DAPI

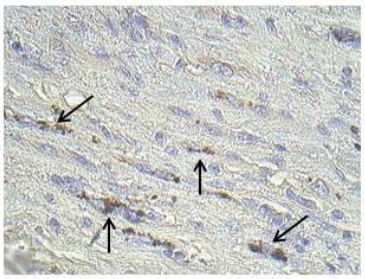
Hybrid Cytochemistry/Immunocytochemistry



(A) (B)

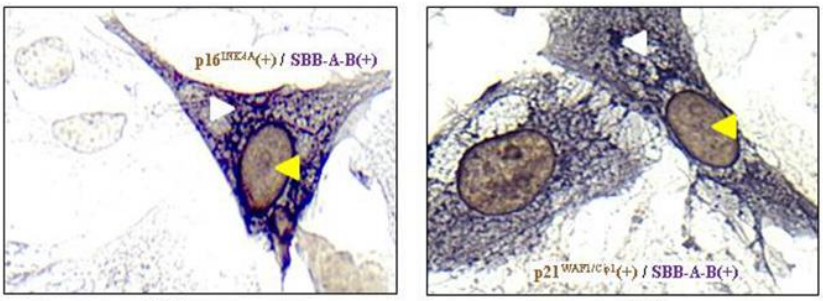
Positive SenTraGor staining in senescent Saos p21 tet-On cells (A) and in late passage human diploid lung fibroblasts (DLFs), undergoing replicative senescence (B). . Chromogen: DAB, Counterstain: Hematoxylin

Hybrid Histochemistry/Immunohistochemistry



Arrows indicate positive SenTraGor staining in: senescent fibroblasts of irradiated human laryngeal cancer tissue. Chromogen: DAB, Counterstain: Hematoxylin

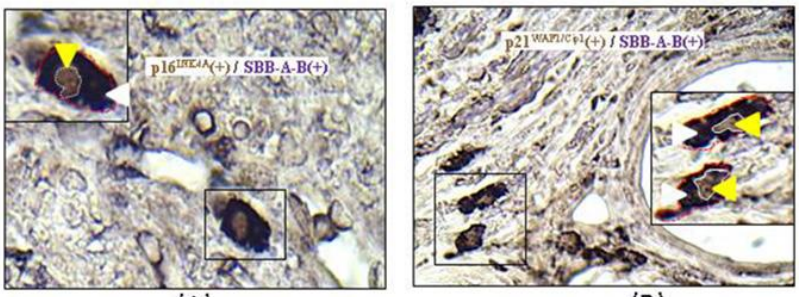
Double staining: Immunocytochemistry and hybrid Histochemistry/Immunohistochemistry



(A) (B)

Representative images from double-staining experiments in irradiated human diploid fibroblasts (DLFs) (**A**) and and induced Saos2-p21$^{WAF1/Cip1}$ cells (**B**). DAB IHC-brown color for p16^{INK4A} and p21$^{WAF1/Cip1}$, respectively (yellow arrowheads) in senescent cells concurrently positive with Sentragor™, visualized with the BCIP/NBT chromogenic hybrid Histo-IHC reaction (dark blue perinuclear and cytoplasmic color).

Double staining: Immunohistochemistry and hybrid Histochemistry/Immunohistochemistry



(A) (B)

Representative images from double-staining experiments in mouse models (K-ras V12-induced lung adenoma) (**A**) and human clinical samples (irradiated breast samples) (**B**), showing nuclear p16INK4Aorp21WAF1/Cip1expression (DAB IHC-brown color: yellow arrowheads) in senescent cells that are concurrently positive with the GL13 compound, visualized with the BCIP/NBT chromogenic hybrid Histo-IHC reaction (dark blue perinuclear and cytoplasmic color: white arrowheads; red dashed line: cell perimeter; white dashed line: nuclear perimeter.

Use

The reagent is for research use.

Protocols

For detailed protocols you can contact us at sentragor@labsupplies.gr or visit our website www.sentragortech.com

9 Antiopis Street, 17343 Athens, Greece Tel:(+30) 2107294603, Fax:(+30) 2107294606, e-mail: info@labsupplies.gr