Datasheet for ABIN2344914

96-well Cellular Senescence Assay (SA β-Gal Activity)

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Overview

Quantity:	120 tests
Reactivity:	Mammalian
Application:	Cellular Assay (CA)

Product Details

Sample Type:	Cell Samples
Detection Method:	Fluorometric
Components:	1. 2X Cell Lysis Buffer : One 10 mL bottle 2. 2X Reaction Buffer : One 10 mL bottle 3. SA-ß-Gal Substrate (20X) : One 300 µL amber tube 4. Stop Solution : One 25 mL bottle 2
Material not included:	1. Senescent cells or tissue samples 2. 37 °C Incubator 3. β-mercaptoethanol 4. 96-well plate suitable for a fluorescence plate reader 5. 96-well Fluorometer 6. Protein Assay Reagents

Target Details

Background:	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

Target Details

ability of the cells to undergo population doubling. Senescent cells are characterized by an irreversible G1 growth arrest involving the repression of genes that drive cell cycle progression and the upregulation of cell cycle INK4a CIP1 inhibitors like p16 , p53, and its transcriptional target, p21 . 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 96-well Cellular Senescence Assay Kit provides an easy-to-use and efficient method to determine the cellular senescence by measuring SA-ß-Gal activity using a fluorometric substrate. This quantitative assay uses cell lysate for both SA-β-galactosidase activity determination and normalization of samples containing different cell numbers. Each Trial Size kit provides sufficient quantities to perform up to 24 assays in a 96-well plate.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Measure activity of senescence-associated ß-galactosidase • Quantitative results in a fluorescence plate reader
Reagent Preparation:	1X Cell Lysis Buffer: Prepare a 1X Cell Lysis Buffer by diluting the provided 2X stock 1:2 in ddH2O. Store the diluted solution at room temperature for up to six months. Immediately before use, add proper amount of proteinase inhibitors such as PMSF. 2X Assay Buffer: Immediately before use, add β-mercaptoethanol to 2X Reaction Buffer at a final concentration of 10 mM and dilute 20X SA-ß-Gal Substrate to 1X with 2X Reaction Buffer containing 10 mM β-mercaptoethanol • Don't store 2X Assay Buffer. Reagent 96-well 24-well 6-well 10 cm 1X Cell Lysis Buffer 100 µL 400 µL 1000 µL 1500 µL Table 1. 1X Cell Lysis Buffer Required per Well for Various Culture Plates.
Assay Procedure:	1. Aspirate the medium from the senescent cells. 2. Wash the cells once with 200 µL of cold 1X PBS and aspirate the wash. 3. Add 100 µL of cold 1X Cell Lysis Buffer (see the table above for the required amount of 1X Cell Lysis Buffer of other plate formats). Incubate at 4 °C for 5 minutes. Transfer the whole

Application Details

lysate to a microcentrifuge tube and centrifuge 10 minutes at 4 °C. Collect supernatant as cell lysate.

4. (optional) Determine the total protein concentration of each cell lysate sample by protein assay such as Pierce's BCA protein Assay.
5. Transfer 50 µL of the cell lysate to a 96-well plate. Add 50 µL of freshly prepared 2X Assay Buffer. Incubate the wells at 37 °C protected from light for 1- 3 hr.
6. Remove 50 µL of the reaction mixture to a 96-well plate suitable for fluorescence measurement. Stop the reaction by adding 200 µL of Stop solution.
7. Read fluorescence with a fluorescence plate reader at 360 nm (Excitation) / 465 nm (Emission). 3

Restrictions:	For Research Use only

Handling

Storage:	RT/-20 °C
Storage Comment:	Store SA-β-gal substrate solution protected from light at -20°C. Store all other components at room temperature.

Publications

Product cited in:

Hu, Sung, Jessen, Thibault, Finkelstein, Khan, Sacaan: "Mechanistic Investigation of Bone Marrow Suppression Associated with Palbociclib and its Differentiation from Cytotoxic Chemotherapies." in: **Clinical cancer research : an official journal of the American Association for Cancer Research**, (2016) (PubMed).

Kim, Lee, Ko, Chun, Kim, Sung, Koo, Yoo: "Cell culture density affects the proliferation activity of human adipose tissue stem cells." in: **Cell biochemistry and function**, Vol. 34, Issue 1, pp. 16-24 , (2016) (PubMed).

Platas, Guillén, Gomar, Castejón, Esbrit, Alcaraz: "Anti-senescence and Anti-inflammatory Effects of the C-terminal Moiety of PTHrP Peptides in OA Osteoblasts." in: **The journals of gerontology. Series A, Biological sciences and medical sciences**, Vol. 72, Issue 5, pp. 624-631 , (2016) (PubMed).

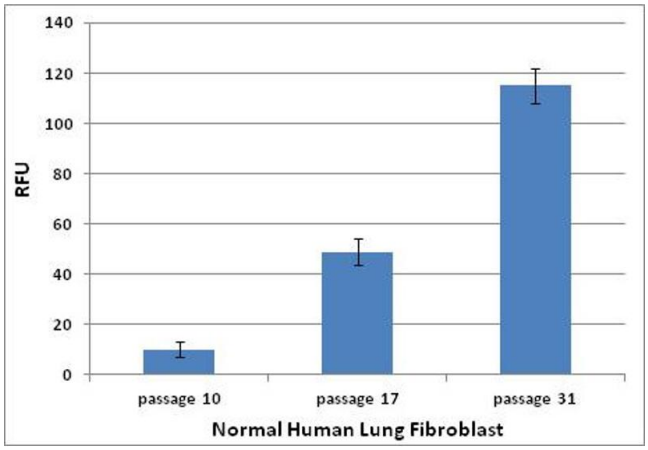
Zhang, Gong, Wang, Chen, Lim, Dolata, Chen: "Cryptosporidium parvum infection attenuates the ex vivo propagation of murine intestinal enteroids." in: **Physiological reports**, Vol. 4, Issue 24, (2016) (PubMed).

Publications

Shimizu, Kanno, Sugiyama, Ohata, Araki, Kishikawa, Kimura, Yamamoto, Kodama, Kihira, Tazuma: "Cholangiocyte senescence caused by lysophosphatidylcholine as a potential implication in carcinogenesis." in: **Journal of hepato-biliary-pancreatic sciences**, Vol. 22, Issue 9, pp. 675-82, (2015) (PubMed).

There are more publications referencing this product on: Product page

Images



Cellular Assay

Image 1. SA-ß-Gal activity in Senescent Human Lung Fibroblast HFL-1 Cells. Normal HFL-1 cells with different passage numbers were lysed. Lysates were allowed to incubate with SA-ß-Gal Substrate for 1 hr at 37°C.