

Cellular Senescence Kit Red

Catalog# / Size	421934 / 100 tests
Regulatory Status	RUO
Other Names	Senescence-associated beta-galactosidase
Description	Cellular senescence is a state of stable cell cycle arrest under which cells remain metabolically active, but no longer divide and do not respond to growth-promoting stimuli. It is triggered by a variety of cellular stressors, including environmental factors like ionizing radiation or exposure to chemotherapeutic drugs, oxidative stress, DNA damage, mitochondrial dysfunction, and oncogene activation. Senescence-Associated β -galactosidase (SA- β -gal), a result of altered lysosomal activity, is a hallmark of cellular senescence. Cellular Senescence Kit Red uses a fluorogenic beta-Gal substrate that readily enters into live cells, where it gets cleaved by SA- β -gal inside cells, generating strong red fluorescence. It provides higher sensitivity compared to X-gal staining, a colorimetric method widely available and used to detect Senescence-associated beta-galactosidase (SA-beta-gal) in senescent cells.

Kit Details

Kit Contents

- 1 vial of lyophilized 100X Senescence Probe Red
- 1 bottle (20 mL) of Senescence Assay Buffer
- 1 vial (100 μ L) of DMSO

Product Details

Verified Reactivity	Mouse, Human
Formulation	Each kit contains 1 vial of lyophilized 100X Senescence Probe Red, 1 bottle (20 mL) of Senescence Assay Buffer, 1 vial (100 μ L) of DMSO.
Storage & Handling	The kit should be stored at $< -15^{\circ}\text{C}$. All unused stock solutions should be divided into single-use aliquots and stored at $< -15^{\circ}\text{C}$ after preparation. Avoid repeated freeze-thaw cycles.
Application	FC - Quality tested Live cell imaging - Verified
Recommended Usage	Please refer to detailed protocol in application notes
Application Notes	<p><u>Components:</u></p> <ul style="list-style-type: none">• 1 vial of lyophilized 100X Senescence Probe Red (beta-D-galactopyranoside) (Store at $< -15^{\circ}\text{C}$ and minimize light exposure. All unused stock solutions should be stored as single-use aliquots and stored at $< -15^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.)• 1 bottle (20 mL) of Senescence Assay Buffer (Store at $< -15^{\circ}\text{C}$)• 1 vial (100 μL) of DMSO (Store at $< -15^{\circ}\text{C}$) <p><u>Required Materials Not Included:</u></p> <ul style="list-style-type: none">• Buffer: PBS <p><u>Detection/Imaging Guidelines:</u></p> <ul style="list-style-type: none">• Fluorescence microscope filter set: Cyanine3/TRITC filter set, recommend black wall/clear bottom plate• Flow cytometry channel:<ul style="list-style-type: none">• Excitation: 488 nm laser• Emission: 575/26 nm filter• Instrument specification(s): PE channel

Assay Protocol:

This kit provides enough reagents for 100 tests. A test is defined as 100 μL of Senescence Probe Red working solution for up to 1×10^6 cells.

1. Preparation of stock solutions:

1. Add 100 μL of DMSO into lyophilized 100X Senescence Probe Red and mix well.

Note: Store the unused Senescence Probe Red stock solution at -20°C in single use aliquots.

2. Preparation of working solutions:

1. Dilute 10 μL of 100X Senescence Probe Red stock solution with 1 mL of Senescence Assay Buffer to make Senescence Probe Red working solution. This can be used for 10 tests; each test will need 100 μL Senescence Probe Red working solution.

Note: Senescence Probe Red working solution should be used promptly.

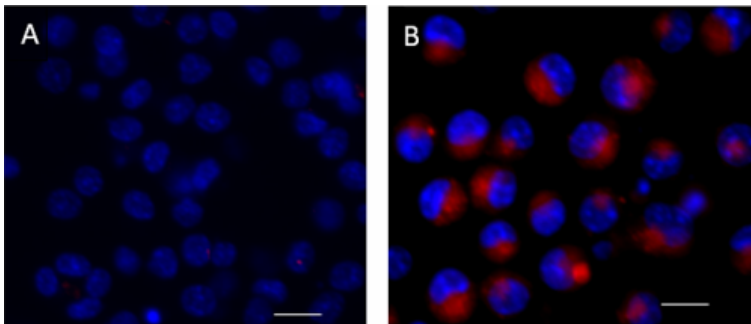
Experimental Protocol:

1. Treat your cell samples under desired experimental conditions. **Note:** For selectively tracking β -Gal in live cells, cells can be treated with Bafilomycin A1 for blocking endogenous β -Gal. Optimum concentration of Bafilomycin A1 may vary depending on cell type (suggested range is 100 nM-10 mM).
2. Gently wash the cells with Cell Staining Buffer (Cat. No. 420201) or PBS.
3. Add 100 μL Senescence Probe Red working solution for up to 1×10^6 cells (for higher cell amounts, scale up accordingly). And incubate the samples at 37°C incubator for 15-45 minutes.
4. Gently remove the Senescence Probe Red solution from cells and wash twice with Cell Staining Buffer (Cat. No. 420201) or PBS.
5. Analyze samples: For flow cytometry, resuspend the cells in the Senescence Assay Buffer and monitor the fluorescence intensity on a flow cytometer using a 575/26 nm filter (PE channel). For live cell imaging, observe cells with inverted fluorescence microscope using a Cyanine3/TRITC filter set.

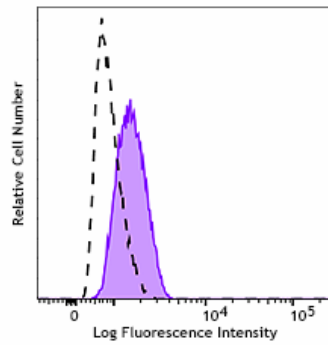
Antigen Details

Distribution	Cytoplasmic
Function	Cellular Senescence activity
Biology Area	Cell Biology, Cell Cycle/DNA Replication, Cell Proliferation and Viability
Molecular Family	Enzymes and Regulators
Gene ID	NA

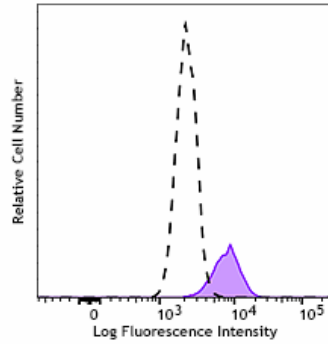
Product Data



Live-cell fluorescence imaging of RAW264.7 untreated (panel A) and treated with Camptothecin (panel B) for 4 days to induce cellular senescence. Cells were then stained with Cellular Senescence Kit Red (red) and nuclei were stained with Hoechst. Images were captured using a 60X objective. Scale bar: $20\mu\text{m}$



RAW264.7 untreated (open histogram) or treated with Camptothecin (filled histogram) for 4 days to induce cellular senescence. Cells were then stained with Cellular Senescence Kit Red and analyzed by flow cytometry.



U937 untreated (open histogram) or treated with Camptothecin (filled histogram) for 3 days to induce cellular senescence. Cells were then stained with Cellular Senescence Kit Red and analyzed by flow cytometry.

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