Senescence Detection Kit I

PromoKine

Detection of senescence marker in cultured cells and tissue sections

Instruction Manual

Catalog Number	PK-CA577-K320
Description	Senescence is thought to be a tumor suppressive mechanism and an underlying cause of aging. Senescence represents an arrested state in which the cells remain viable, but are not stimulated to divide by serum or passage in culture. Senescent cells display increase of cell size, senescence-associated expression of β-galactosidase (SA-β-Gal) activity, and altered patterns of gene expression. The PromoKine Senescence Detection Kit is designed to histochemically detect SA-β-Gal activity, a known characteristic of senescent cells, in cultured cells and tissue sections. The SA-β-Gal is present only in senescent cells and is not found in presenescent, quiescent or immortal cells.
Quantity	250 assays

Kit Components

Components	Quantity	Color Code
Fixative Solution (1X)	125 ml	NM
X-Gal (150 mg, lyophilized)	1 vial	Green
Staining Solution (1X)	125 ml	WM
Staining Supplement (100X)	1.5 ml	Red

Applications / Assay Protocol

A. General Consideration & Reagent Preparations:
The following protocol is designed for 12-well culture plates. For using larger plates, increase the volume accordingly (e.g., For 6-well plate, double the volume).

- Prepare 1X PBS Solution (not provided). Prepare 3 ml per well.
- Prepare X-gal Solution: Dissolve 20 mg X-gal in 1 ml DMSO or DMF (N-N dimethylformamide, not provided) to prepare a 20X stock solution. Excess X-gal solution can be stored at –20°C (protected from light) for one month. Always use polypropylene container or glass to make and store X-gal. Do not use polystyrene.
- Fixative Solution (1X), Staining Solution (1X), and Staining Supplement (100X) can be stored at 4°C.
- Staining Solution and Staining Supplement: If precipitation occurs, simply warm up the solution to 37°C to solubilize the precipitates. If precipitation persists, centrifuge the vial and use the supernatant for staining.

B. Staining Protocol:

1. Remove culture medium and wash cells once with 1 ml of 1X PBS.
2. Fix the cells with 0.5 ml of Fixative Solution for 10-15 minutes at room temperature.
3. While the cells are in the Fixative Solution, prepare the Staining Solution Mix. Using polypropylene plastic tubes only. Prepare enough solution for the number of wells to be stained. For each well, prepare:
    - 470 µl of Staining Solution
    - 5 µl of Staining Supplement
    - 25 µl of 20 mg/ml X-gal in DMF
4. Wash the cells two times with 1 ml of 1X PBS.
5. Add 0.5 ml of the Staining Solution Mix to each well. Cover the plate. Incubate overnight at 37°C.
6. Observe cells under a microscope for development of blue color (200X total magnification).
7. For long-term storage of the stained plates, remove the Staining Solution and overlay the cells with 70% glycerol. Store at 4°C.

Storage & Stability

Store kit at 4°C or –20°C and protect from light. Store reconstituted X-gal at –20°C.

PromoCell GmbH
Sickingenstr. 63/65
69126 Heidelberg
Germany

North America 1 – 866 – 251 – 2860 (toll free)
Deutschland 0800 – 776 66 23 (gebührenfrei)
France 0800 90 93 32 (ligne verte)
United Kingdom 0800 – 96 03 33 (toll free)
Other Countries +49 6221 – 649 34 0

Email: info@promokine.info
www.promokine.info

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