



Cell Senescence Assay

Cat. No. CB011
(50 Tests in 35mm plate)

Description

Normal mammalian cells divide for a limited number of population doublings and eventually enter an arrested state in which the cells remain alive, but do not proliferate in response to mitogens, and assume a characteristic enlarged, flattened morphology. This process is senescence and thought to be a tumor suppressive mechanism and underlying cause of aging. Senescence-associated β -galactosidase (SA- β -gal) is a widely used biochemical maker for assessing senescence in cultured cells. The Cell Senescence Assay provide an easy-to-use method to detect SA- β -gal by staining cells with 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal) at pH 6.0, a pH condition that suppress lysosomal β -galactosidase activity sufficiently to ensure that non-senescent cells remain unstained.

Kit Components

100X Fixing Solution: One tube, 1.5 mL of 25% Glutaraldehyde

Staining Solution A: One tube, 1.5 mL of 500 mM Potassium Ferrocyanide

Staining Solution B: One tube, 1.5 mL of 500 mM Potassium Ferricyanide

Staining Solution C: Three tubes, 1.5 ml of 1 M Citrate- Na_2HPO_4 Buffer, pH6.0, 50 mM MgCl_2

Staining Solution D: Two tubes – 2.0 mL of 5 M NaCl in each tube

X-gal Solution: Two tubes – 1.5 mL of 40 mg/mL X-gal in DMF in each tube

Materials Not Supplied

1. PBS
2. 37°C Incubator
3. Light microscope
4. Senescent cells or tissue samples

* Store X-gal solution protected from light at -20°C. Store all other components at 4°C. Crystal deposition, which comes from unreacted X-gal, may be observed after incubation of cells with working staining solution. It can be minimized by pre-filtering the working staining solution with a 0.2 μm filter.

Procedures

A. Preparation of reagents

1. Preparation of working fixing solution: Prepare 1 \times fixing solution by diluting 100X Fixing Solution stock 1:100 in PBS.
2. Preparation of working staining solution: Prepare fresh staining working solution based on the number of samples to be assessed. For each sample in 35 mm plate, prepare the following mixture:

100 μl of X-gal Solution
20 μl of Staining Solution A
20 μl of Staining Solution B
4 μl of Staining Solution C
+ 1856 μl of Staining Solution D
2000 μl of working staining solution

B. Staining protocol

1. Remove culture medium from cells and rinse twice with PBS.
2. Fix cells by incubating with 2 ml of working fixing solution for 3-5 minutes at room temperature.
3. Aspirate working fixing solution and rinse the fixed cells three times with PBS.
4. Add 2 ml of working staining solution to completely cover cells and incubate cells at 37°C, protected from light, for 12-24 hours, blue color should develop in senescent cells.* Examine cells at regular time points to avoid overstaining.
5. After incubation, remove working staining solution and rinse cells twice with PBS, keep the cells in PBS at 4°C. Examine and count the blue stained cells using a light microscope.