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 nonsenescent 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. 37ºC Incubator
3. Light microscope
4. Senescent cells or tissue samples

* Store X-gal solution protected from light at -20ºC. Store all other components at 4ºC. 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× 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 overstraining.
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.