Cell Senescence Assay
Cat. No. 8058, 50 Tests in 35mm plate

Introduction

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, termed senescence, is 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 ScienCell™ 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

Cat. No.	# of vials	Reagent	Quantity	Storage
8058a	1	Staining Solution A	1 ml	4°C
8058b	1	Staining Solution B	1 ml	4°C
8058c	1	Staining Solution C	0.2 ml	4°C
8058d	1	Staining Solution D	100 ml	4°C
8058e	1	X-gal Solution	5 ml	-20°C
8058f	1	100× Fixing Solution	1 ml	4°C

Quality Control

The Cell Senescence Assays are applied to early passage and senescent ScienCell™ Human Renal Proximal Tubular Epithelial Cells (HRPTEpiCs). Data show that the senescent cells show positive SA- β-gal staining while most early passage cells don't (Figure 1).

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.

* 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.

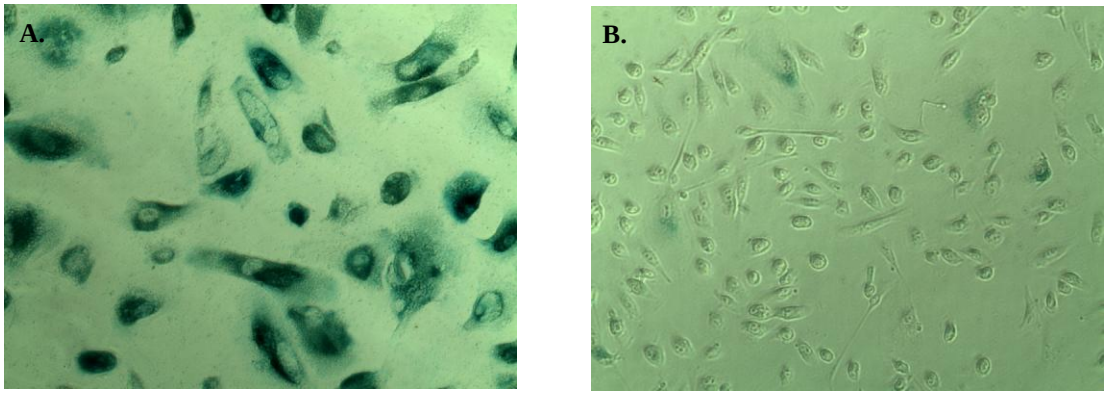

Figure 1. Human renal proximal tubular epithelial cells (HRPTEpiCs) probed with ScienCell™ Senescence Assay. Most senescent cells show positive SA-β-Gal staining (A), while only a few labeled cells can be observed in early passage culture (B).