

cell signaling

Senescent Cells Staining Kit

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Application Notes

- Contains all the reagents required for identifying senescent cells
- Simple and rapid staining procedure based on histochemical stain for β -galactosidase
- Sufficient for at least 100 tests in 3.5 cm cell culture plates

Introduction

Cellular senescence is a progression of events whereby cells move from an actively dividing to a non-dividing state, yet remaining metabolically active. The cellular senescence process is associated with aging and age-related diseases including atherosclerosis, osteoarthritis, immune senescence, skin aging, Alzheimer's dementia and cancer. The decrease in cell division is virtually irreversible and complete. In conjunction with the loss of the ability to divide, changes occur in the morphology, shape, and physical appearance of the cells, and in their pattern of gene expression. Although the cells may remain viable for a long time, at the end of the process cell death usually occurs. The Senescence Cells Staining Kit (Product Code [CS0030](#)) is designed for identifying senescent cells using a simple and rapid staining procedure.

Principle of the assay

The kit is based on a histochemical staining for β -galactosidase activity, at pH 6, which is unique to senescent cells. The activity at pH 6 is easily detectable in senescent cells, but undetectable in quiescent, immortal or tumor cells.¹ As a control, cells are also stained at acidic pH 4 in which eukaryotic lysosomal β -galactosidase is active in all cells.

Procedure

Several types of cells representing normal and senescent cells were used as a model for illustrating the principle of the kit.

Normal cells

- Human Foreskin Fibroblasts (HFF) primary cells at an early passage (passage 5).
- pIND-p53 cells - H 1299 cells stably transfected with Ponasterone A/Muristeron induced p53 gene.²

Senescent cells

- Human Foreskin Fibroblasts (HFF) primary cells at a late passage (passage 28).
- Ponasterone A induced pIND-p53 cells - H 1299 cells stably transfected with Ponasterone A/Muristeron induced p53 gene.² Induction of p53 expression induces senescence in the cells within 4-5 days.

Control cells

- HeLa cells, transfected or non-transfected with a bacterial β -galactosidase reporter plasmid.
- Ponasterone A treated HeLa cells.

Cells were grown in 35-mm tissue culture plates or a 96-well plate and treated according to the procedure illustrated in Figure 1 using the Senescent Cells Staining Kit (Product Code [CS0030](#)). The cells were fixed and incubated at 37 °C

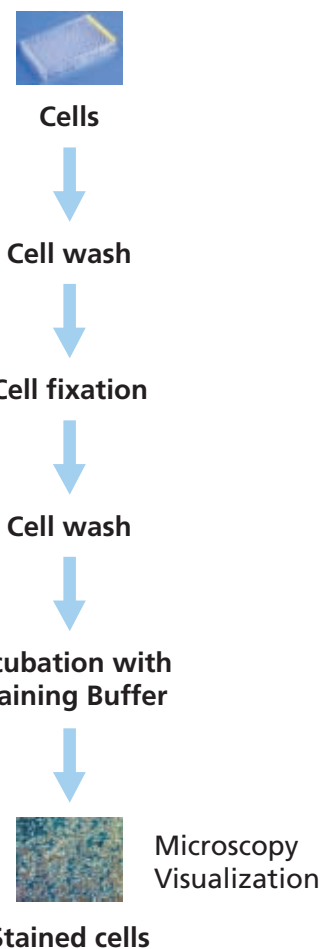


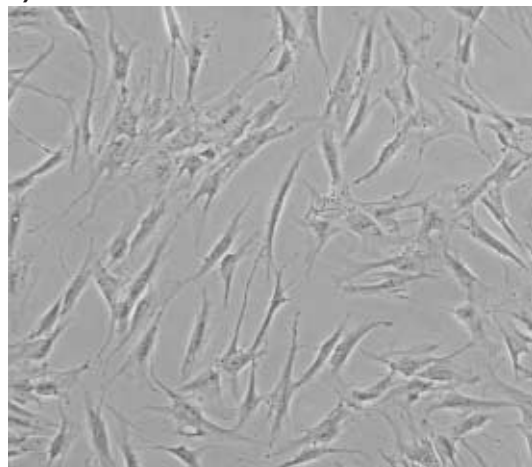
Figure 1. Flow chart of the staining procedure.

Table 1. β -galactosidase activity in various cell types

Cells/pH	HFF Passage 5	HFF Passage 28	Non-induced pIND-p53 H 1299	Induced pIND-p53 H 1299	HeLa	Muristeron- treated HeLa	HeLa β -gal transfected
4	+	+	+	+	+	+	+
6	-	+	-	+	-	-	-
7.5	-	-	-	-	-	-	+

The appearance of a blue color in cells, due to β -galactosidase activity, is indicated by (+). The cells were incubated with an X-gal substrate containing staining buffer at different pHs (4, 6 and 7.5). β -galactosidase staining representing activity specific for senescent cells is highlighted in red.

A)



B)

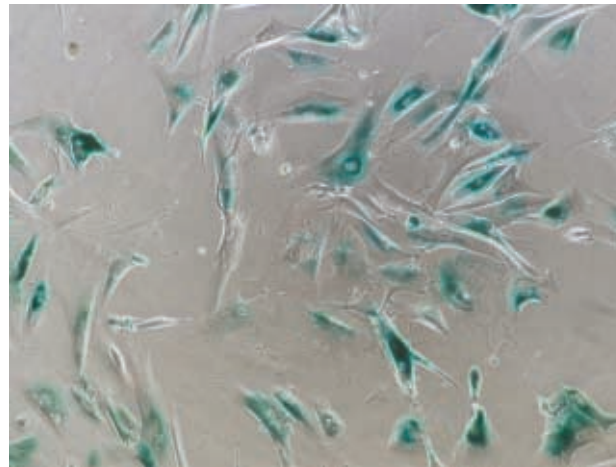


Figure 2. Detection of senescent cells by β -galactosidase staining. Human Foreskin Fibroblasts (HFF) primary cells at an early passage (A: 5 passages) and at a late passage (B: 28 passages) were stained using the Senescent Cell Staining Kit (Product Code [CS0030](#)). The HFF cells at passage 28 show a blue staining indicating that these are senescent.

in a pH 6 staining buffer, containing an artificial β -galactosidase substrate (X-gal). As a control experiment, the fixed cells were stained also with staining buffer at pH 4 at which the lysosomal β -galactosidase is active, or at pH 7.5, at which only the bacterial β -galactosidase in transfected cells is active.

Results

Table 1 summarizes the staining data of the senescent and control cells in pH 4, pH 6 and pH 7.5. As expected, at pH 4 all the cells tested stained blue due to the activity of the mammalian lysosomal β -galactosidase that is active at pH 4. At pH 7.5 only HeLa cells expressing the bacterial β -galactosidase stained blue since this is the optimal pH for the bacterial β -galactosidase. Blue staining at pH 6 was observed only in HFF cells at a late passage (Figure 2B) or in Ponasterone A induced pIND-p53 cells. There was no staining in HFF at an early passage (Figure 2A), in non-induced pIND-p53 cells or in HeLa cells. These results indicate that β -galactosidase staining at pH 6 occurs only in senescent cells.

Discussion

Cleavage of X-gal by β -galactosidase at pH 6.0 leading to a blue staining of cells was found to be specific for senescent cells, while this cleaving activity is not detectable at pH 6.0 in pre-senescent cells whether they are growing or immortal (cell line). Staining of cells using the Senescent Cells Staining Kit (Product Code [CS0030](#)), as described above, allows a fast and easy detection of senescent cells and provides a tool for distinguishing between senescent and pre-senescent cells.

References

- Dimri, G.P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E.E., Linskens, M., Rubelj, I., Pereira-Smith, O., Peacocke, M., and Campisi, J., Biomarker that identifies senescent human cells in culture and in aging skin *in vivo*. *Proc. Natl. Acad. Sci. USA*, **92**, 9363-9367 (1995).
- Wang, Y., Blandino, G., Oren, M., and Givol, D., Induced p53 expression in lung cancer cell line promotes cell senescence and differentially modifies the cytotoxicity of anti-cancer drugs. *Oncogene*, **17**, 923-930 (1998).

Manufactured under license to US Patent Nos. 5,491,069 and 5,795,728.

Ordering Information

Product	Description	Unit
CS0030	Senescence Cells Histochemical Staining Kit	1 kit