

# Senescence $\beta$ -Galactosidase Staining Kit

- Reagents for 125 thirty-five mm wells



## Cell Signaling

TECHNOLOGY™

**Orders** ■ 877-616-CELL (2355)  
orders@cellsignal.com

**Support** ■ 877-678-TECH (8324)  
info@cellsignal.com

**Web** ■ [www.cellsignal.com](http://www.cellsignal.com)

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### Staining Kit Includes:

- **10X Fixative Solution**  
15 ml
- **X-Gal**  
150 mg
- **10X Staining Solution**  
15 ml

- **100X Staining Solution Supplement A:  
500 mM potassium ferrocyanide**  
1.5 ml

- **100X Staining Solution Supplement B:  
500 mM potassium ferricyanide**  
1.5 ml

**Background:** Limited capacity to replicate is a defining characteristic of most normal cells and culminates in senescence, an arrested state in which the cell remains viable but displays altered patterns of gene and protein expression (1). Senescent cells are not stimulated to divide by serum or passage in culture, and senescence invokes a specific cell cycle profile that differs from most damage-induced arrest processes or contact inhibition (2). An enlarged cell size, expression of pH-dependent  $\beta$ -galactosidase activity (3) and an altered pattern of gene expression (4,5) further characterize senescent cells.

**Description:** The Senescence  $\beta$ -Galactosidase Staining Kit\* is designed to histochemically detect  $\beta$ -galactosidase activity at pH 6, a known characteristic of senescent cells (3). The kit includes all reagents necessary for this assay.

**Specificity:** The kit detects only  $\beta$ -galactosidase activity at pH 6 in cultured cells and tissue sections.  $\beta$ -galactosidase activity at pH 6 is present only in senescent cells and is not found in presenescent, quiescent or immortal cells.

**Applications:**  $\beta$ -galactosidase staining

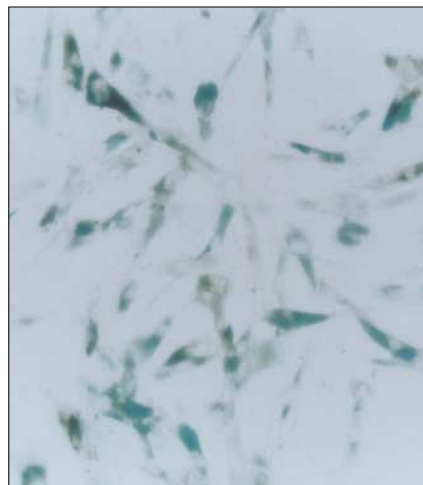
### References:

- (1) Goldstein, S. (1990) *Science* 249, 1129–1133.
- (2) Sherwood, S.W. et al. (1988) *Proc. Natl. Acad. Sci. USA* 85, 9086–9090.
- (3) Dimri, G. et al. (1995) *Proc. Natl. Acad. Sci. USA* 92, 9363–9367.
- (4) Cristofalo, V.J. et al. (1998) *Crit. Rev. Eukaryot Gene Expr.* 8, 43–80.
- (5) Linskens, M.H. et al. (1995) *Nucleic Acid Res.* 23, 3244–3251.

### Companion Products:

$\beta$ -Gal 14B7 Monoclonal Antibody #2372

\* See enclosed Material Safety Data Sheet or refer to our website for further information.



$\beta$ -galactosidase staining at pH 6 on normal WI38 cells at population doubling 29 (left) and senescent WI38 cells at population doubling 36 (right).

## Senescence $\beta$ -Galactosidase Staining Kit Protocol

### Solutions and Reagents

Reagents provided are sufficient to stain 125 thirty-five mm wells.

10X Fixative Solution (15 ml):

20% formaldehyde, 2% glutaraldehyde in 10X PBS.

X-gal (150 mg):

5-bromo-4-chloro-3-indolyl- $\beta$ D-galactopyranoside powder.

10X Staining Solution (15 ml):

400 mM citric acid/sodium phosphate (pH 6.0), 1.5 M NaCl, 20 mM MgCl<sub>2</sub>.

100X Staining Solution Supplement A (1.5 ml):

500 mM potassium ferrocyanide.

100X Staining Solution Supplement B (1.5 ml):

500 mM potassium ferricyanide.

### Materials Supplied by the User

- 1X PBS (Phosphate Buffered Saline):  
1.7 mM KH<sub>2</sub>PO<sub>4</sub>, 5 mM Na<sub>2</sub>HPO<sub>4</sub>, 150 mM NaCl (pH 7.4)
- N-N-dimethylformamide (DMF)
- 70% glycerol (optional)
- 37° C incubator
- Phase contrast or light microscope
- Polypropylene tubes

### Protocol

The following protocol is designed for one 35 mm well of a 6-well plate. Volumes in the procedure should be roughly half that of the tissue culture media (e.g., 1 ml for 35 mm wells/plates, 2.5 ml for 60 mm plates and 5 ml for 100 mm plates).

### Setup

1. Prepare a 1X PBS solution (not provided). Prepare 6 ml per 35 mm well.
2. Dilute the 10X Staining and 10X Fixative Solutions with distilled water to make 1X solutions. You will need 930  $\mu$ l of 1X Staining Solution and 1 ml of 1X Fixative Solution per 35 mm well.
3. Dissolve 20 mg X-gal in 1 ml DMF to prepare a 20X stock solution. Excess X-gal solution can be stored at -20°C in a light resistant container for one month. Always use *polypropylene* plastic or glass to make and store X-gal solutions. Do not use polystyrene.

### Procedure

1. Remove the growth medium from the cells and wash the plate once with 2 ml 1X PBS.
2. Fix the cells with 1 ml 1X Fixative Solution for 10–15 minutes at room temperature.
3. While the plate is in the Fixative Solution, prepare the Staining Solution. Use *polypropylene* plastic only.
  - a. 930  $\mu$ l Staining Solution
  - b. 10  $\mu$ l Staining Supplement A
  - c. 10  $\mu$ l Staining Supplement B
  - d. 50  $\mu$ l 20 mg/ml X-gal in DMF
4. Wash the well *twice* with 2 ml 1X PBS.
5. Add 1 ml Staining Solution mix to the plate. Incubate overnight at 37°C.
6. Check the cells under a microscope (200 x total magnification) for development of blue color.
7. For long-term storage of stained wells/plates, remove the Staining Solution and overlay the cells with 70% glycerol. Store at 4°C.