#9860 Store at -20°C

Senescence β-Galactosidase Staining Kit

1 Kit
(125 x 35 mm wells)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Item #	Kit Quantity
10X Fixative Solution	11674	15 ml
10X Staining Solution	11675	15 ml
100X Solution A	11676	1.5 ml
100X Solution B	11677	1.5 ml
X-Gal	11678	150 mg

Description: The Senescence β-Galactosidase Staining Kit is designed to conveniently provide reagents needed to detect β-galactosidase activity at pH 6, a known characteristic of senescent cells. Papers have published using this kit in both cultured cells and frozen tissue.

NOTE: DMF (Dimethylformamide) is required as a solvent for the X-gal, but is not supplied with the kit. CST recommends product DMF #12767 as the solvent of choice. The product is 99.9% pure and its convenient 10 ml size is more than enough material to dissolve the 150 mg of X-Gal supplied in a single #9860 kit.

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 (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.

Background References:

(1) Goldstein, S. (1990) *Science* 249, 1129-1133.

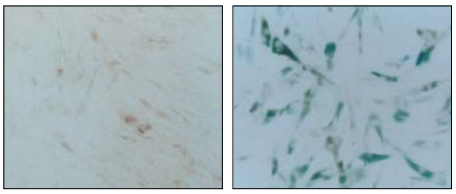
(2) Sherwood, S. W. et al. (1988) *Proc. Natl. Acad. Sci. , USA* 85, 9086-9090.

(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.

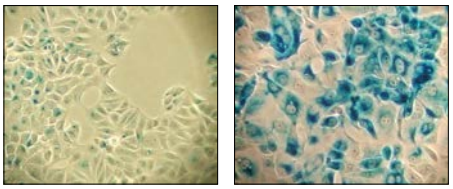
(5) Linskens, M. H. et al. (1995) *Nucleic Acid Res.* 23, 3244-3251.

Replicative Senescence



β-Galactosidase staining at ph 6.0 on normal WI38 cells at population doubling 29 (left) and senescent WI38 cells at population doubling 36 (right).

Premature Senescence



β-Galactosidase staining at pH 6.0 on untreated MCF-7 cells (left) and senescent MCF-7 cells treated with etoposide #2200 (12.5 μM, 24 hr) and allowed to recover for 4 days (right).

Application References:

Baker, D. J. et al. (2004) BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. *Nat. Genetics* 36, 744-749. Application: IHC-FL (floating/frozen).

Su, D. et al. (2009) BMP4-Smad signaling pathway mediates adriamycin-induced premature senescence in lung cancer cells. *J Biol Chem* 284, 12153-64.

Swarbrick, A. et al. (2008) Id1 cooperates with oncogenic Ras to induce metastatic mammary carcinoma by subversion of the cellular senescence response. *Proc Natl Acad Sci U S A* 105, 5402-7. Application: IHC-FL (floating/frozen).

Efeyan, A. et al. (2007) Induction of p53-dependent senescence by the MDM2 antagonist nutlin-3a in mouse cells of fibroblast origin. *Cancer Res* 67, 7350-7.

Boucher, M.J. et al. (2004) Dual role of MEK/ERK signaling in senescence and transformation of intestinal epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 286, G736-46.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#9860

Senescence β-Galactosidase Cell Staining Protocol

A Solutions and Reagents

Reagents provided are sufficient to stain 125 x 35 mm wells.

Supplied Reagents

1. 10X Fixative Solution #11674
2. 10X Staining Solution #11675
3. 100X Solution A #11676
4. 100X Solution B #11677
5. X-Gal #11678

Additional Reagents (Not Supplied)

1. 1X PBS
2. DMF (Dimethylformamide) #12767
3. Polypropylene tubes
4. 37°C dry incubator (No CO_2)
5. Phase contrast or light microscope
6. 70% glycerol (optional)

B Solution Preparation

NOTE: All solutions should be prepared just prior to use.

Volumes are for one 35 mm well of a 6-well plate. Volumes in the procedure should be approximately half that of the tissue culture media. (e.g. 1 ml for 35 mm well/plates containing 2 ml of media, 2.5 ml for 60 mm plates containing 5 ml of media, and 5 ml for 100 mm plates containing 10 ml of media).

1. **PBS:** Prepare at least 6 ml 1X PBS per 35 mm well
2. **Fixative Solution:** Dilute the 10X Fixative Solution to a 1X solution with distilled water. You will need 1 ml of the 1X solution per 35 mm well.
3. **Staining Solution:** Redissolve the 10X Staining Solution by heating to 37°C with agitation. Dilute the 10X staining solution to a 1X solution with distilled water. You will need 930 ul of the 1X Staining Solution per 35 mm well.
4. **X-Gal:**
   IMPORTANT: Always use **polypropylene** plastic or glass to make and store X-gal. Do not use polystyrene.
   Dissolve 20 mg of X-gal in 1 ml DMF to prepare a 20 mg/ml stock solution. Excess X-gal solution can be stored in -20°C in a light resistant container for up to one month.
5. **β-Galactosidase Staining Solution:** For each 35 mm well to be stained, combine the following in a **polypropylene** container:
   a. 930 µl 1X Staining Solution (See step 3)
   b. 10 µl 100X Solution A
   c. 10 µl 100X Solution B
   d. 50 µl 20mg/ml X-gal stock solution (see Step 4)

   IMPORTANT: Due to variations in water pH, please be sure that the β-Galactosidase Staining Solution has a final pH of 6.0 (A pH 5.9-6.1 is acceptable). pH differences can affect staining: A low pH can result in false positives and high pH can result in false negatives. If necessary, use HCl or NaOH to lower or raise pH, respectively.

C Procedure:

1. Remove growth media from the cells.
2. Rinse the plate one time with 1X PBS (2 ml or a 35 mm well plate, or match volume of media)
3. Add 1 ml of 1X Fixative Solution to each 35 mm well. Allow cells to fix for 10-15 min at room temperature.
4. Rinse the plate two times with 1X PBS
5. Add 1 ml of the β-Galactosidase Staining Solution to each 35 mm well (see Solution Preparation Step 5).
   Important: Seal plate with parafilm to prevent evaporation. Evaporation can cause crystals to form.
6. Incubate the plate at 37°C at least overnight in a dry incubator (no CO_2).
   Note: The presence of CO_2 can cause changes to the pH which may affect staining results.
7. While the β-galactosidase is still on the plate, check the cells under a microscope (200X total magnification) for the development of blue color.
8. For long-term storage of the plates, remove the β-Galactosidase staining solution and overlay the cells with 70% glycerol. Store at 4°C.