User Protocol

Revision	07-August-2008 JSW
Form	250 Tests
Format	12-well plate
Detection method	Colorimetric
Storage	Upon arrival store the entire contents of the kit at -20°C.
Intended use	The Calbiochem® Senescence Detection Kit is useful for the histochemical detection of senescent cells. The failure of cells to become senescent may lead to tumor progression. The mechanism of this is believed to be the increased lysosomal content of senescent cells resulting in an increase of the lysosomal enzyme β-galactosidase.
Background	Senescence is thought to be a tumor suppressive mechanism and an underlying cause of aging. It represents an arrested state in which the cells remain viable, but not stimulated to divide by serum or by passage in culture. Senescent cells display an increase in cell size, pH-dependent expression of β-galactosidase (β-Gal) activity, and altered patterns of gene expression.
Principles of the assay	The Calbiochem® Senescence Detection Kit is designed to histochemically detect β-Gal activity in cultured cells and in tissue sections at pH 6.0, which is a known characteristic of senescent cells. β-Gal at pH 6.0 is present only in senescent cells and is not found in presenescent, quiescent, or immortal cells.
Materials provided	• **Fixative Solution (Kit Component No. JA7633-125ML):** 1 vial, 125 ml • **X-Gal (Kit Component No. JA7634-150MG):** 1 vial, 150 mg • **Staining Solution (Kit Component No. JA7635-125ML):** 1 vial, 125 ml

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	• **Staining Supplement (Kit Component No. JA7636-1.5ML):** 1 vial, 1.5 ml
Materials Required but not provided	• PBS • DMF
Reagent preparation	• **PBS:** Prepare a 1X PBS solution (not provided). Use at least 3 ml per well. • **X-gal:** Dissolve 20 mg X-gal in 1 ml of DMF (N-N-dimethylformamide, not provided) to prepare a 20X stock solution. Aliquot and freeze (-20°) the unused X-gal solution. This stock solution is stable for up to 1 month at -20°C (protect from light). Always store X-gal in a polypropylene or glass container. Do not use polystyrene. • **Staining Solution Mix:** While cells are incubating in Fixative Solution (step 2 below), prepare Staining Solution Mix in a polypropylene plastic tube by adding 470 µl of Staining Solution, 5 µl Staining Supplement, and 25 µl of 20 mg/ml X-gal in DMF.
Detailed protocol	**Staining** Note: this procedure is described for one well of a 12-well culture plate. For a large plate, increase the volume proportionally. Please review the entire procedure before starting experiments. 1.Remove culture medium and wash cells once with 1 ml 1X PBS. 2. Fix cells with 0.5 ml of Fixative Solution at room temperature for 10 to 15 min. 3. Wash the cells twice with 1 ml 1X PBS. 4. Add 0.5 ml Staining Solution Mix to each well. Incubate at 37°C overnight. 5. Observe cells under a microscope for development of blue color (approximately 200X total magnification). 6. For long-term storage of the stained plates, remove the staining solution mix and overlay the cells with 70% glycerol. Store at 4°C.
Application references	Funasaka, T., et al. 2007. *J. Biol. Chem.* **282,** 36362. Chen, Z., et al. 2005. *Nature* **436,** 725.

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