

ab65351 - Senescence Detection Kit

For the rapid, sensitive and accurate measurement of Senescence in cell and tissue cultures.

[View kit datasheet: www.abcam.com/ab65351](http://www.abcam.com/ab65351)

(use www.abcam.cn/ab65351 for China, or www.abcam.co.jp/ab65351 for Japan)

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

1. Overview

Senescence is thought to be a tumor suppressive mechanism and an underlying cause of aging. Senescence represents an arrested state in which the cells remain viable, but not stimulated to divide by serum or passage in culture. Senescent cells display increase of cell size, senescence-associated expression of β -galactosidase (SA- β -Gal) activity, and altered patterns of gene expression.

Abcam's Senescence Detection Kit is designed to histochemically detect SA- β -Gal activity in cultured cells and tissue sections, a known characteristic of senescent cells. The SA- β -Gal is present only in senescent cells and is not found in pre-senescent, quiescent or immortal cells.

2. Components and Storage

A. Kit Components

Item	Quantity
Fixative Solution III/1X Fixative Solution	125 mL
X-Gal (150 mg, Lyophilized)	1 vial
Staining Solution I/1X Staining Solution	125 mL
100X Staining Supplement	1.5 mL

Store X-Gal (150 mg, Lyophilized) at -20°C, protected from light. Store reconstituted X-Gal at -20°C.

Store Fixative Solution III/ 1x Fixative Solution at -20°C, If precipitation occurs, simply warm up the solution to 37°C to solubilize the precipitates.

Staining Solution I/1X Staining Solution and 100X Staining Supplement can be stored at 4°C. If precipitation occurs, simply warm up the solution to 37°C to solubilize the precipitates.

All components supplied are stable for 1 year.

For long-term storage of the stained plates, remove the Staining Solution I/Staining Solution and overlay the cells with 70% glycerol. Store at -20°C.

B. Additional Materials Required

- Pipettes and pipette tips
- PBS Solution
- DMSO or DMF (N-N-dimethylformamide)
- 12 well plate
- Light microscope

3. Assay Protocol

The following protocol is designed for each well in a 12-well plate. For using a larger plate, increase the volume proportionally (e.g., for 6-well plate, double the volume).

1. Reagent Preparation:

- Prepare 1X PBS Solution (not provided). Prepare 3 mL per well.
- Prepare X-gal Solution: Weigh 20 mg X-gal, dissolve in 1 mL DMSO or DMF (N-N-dimethylformamide, not provided) to prepare a 20X stock solution. Excess X-gal solution can be stored at -20°C (protected from light) for one month. Always use a polypropylene container or glass to make and store the X-gal. Do not use polystyrene.

2. Sample Preparation:

- Remove culture medium and wash cells once with 1 mL of 1X PBS.
- Fix the cells or frozen tissue sections with 0.5 mL of Fixative Solution III/Fixative Solution for 10 - 15 min at room temperature.

Staining Solution Mix: While the cells are in the Fixative Solution III/Fixative Solution, prepare the Staining Solution Mix using a polypropylene plastic tube only. Prepare enough solution for the number of wells to be stained.

For each well, prepare:

Staining Solution I/Staining Solution	470 µL
Staining Supplement	5 µL
20 mg/ml X-gal in DMF	25 µL

Wash the cells twice with 1 ml of 1X PBS.

4. Add 0.5 ml of the Staining Solution Mix to each well. Cover the plate. Incubate plate at 37°C (1 hour – overnight incubation).

NOTE: CO₂ levels found in general 37°C incubators will lower the pH of the Staining Solution I/staining solution hereby affecting the color development. We suggest putting the plate inside a Ziplock® re-sealable bag to avoid any effect from the CO₂.

5. Observe the cells under a microscope for development of blue color (200X total magnification).

4. Frequently Asked Questions

Question	Answer
Can frozen tissue sections be used with this kit?	The kit has been used for skin sections successfully. Briefly, the tissue was frozen in liquid nitrogen, and mounted in OCT. The thin sections (4 µm) were cut, mounted onto glass slides, fixed in 1% formalin in PBS for 1 min at room temp., washed in PBS, immersed overnight in beta-Gal Staining Solution I/staining solution. Then you can view under bright field at 100-200X. The staining results can be found in the following article (The reference is also a principal reference describing the senescence marker) <i>Dimri, G.P., et al. (1995) PNAS 92:9363-9367.</i>
Can paraffin tissue sections be used with this kit?	This has not been determined yet.
Reference article describing the senescence marker?	<i>Dimri, G.P., et al. (1995) PNAS 92:9363-9367</i>
Which cells or tissue have been tested?	Skin tissue section (frozen);
Does this kit detect transient expression of p53 (3-5 days) or longer term expression?	The Senescence Detection Kit (ab65351) will detect senescent cells. If the p53 expressing cells become senescent, then the kit should detect. It does not matter what causes senescence, but as long as cells become senescent, the kit will detect.

Why are some crystals formed after leaving overnight?	These crystals are salt crystals formed due to the solvent evaporation. Our recommendation is to keep the plate sealed when is left overnight.
What if Staining Solution I/Staining Solution and Staining Supplement show precipitates?	Simply warm up the solution to solubilize the precipitates. If precipitation persists, centrifuge the vial and use the supernatant.

5. Technical Support

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