Senescence Detection Kit

(Colorimetric Readout)

Ordering info

TBK0525, 100 assays

Description

Senescence Detection Kit enables reliable identification of senescent cells *in vitro* by detecting the enzymatic activity of lysosomal senescence-associated **β-galactosidase (SA-β-gal)**, a widely accepted specific biomarker of cellular senescence. This assay is based on the hydrolysis of substrate (*5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside*), a chromogenic substrate, which yields an easily visible blue precipitate under light microscopy.

β-Galactosidase activity is significantly increased in senescent cells, allowing their clear identification even in heterogeneous cultures. The enzyme hydrolyzes the substrate into a colored product that accumulates within the cells, enabling visual detection of them.

Features

- **Direct visual detection of senescence**, through the formation of a blue precipitate.
- **Compatible** with post-treatment analysis.
- **Highly specific**, differentiation between SA-β-gal (pH 6.0) and basal lysosomal activity.

Applications

- Evaluation of anti-senescence therapies (senolytics).
- Characterization of cells at the Hayflick limit.
- Phenotypic profiling of tumor or primary cell cultures.

Kit Components

Components	TBK0525
Substrate X-Gal [a]	6 x 20 mg
Fixative Solution 10x [b]	15 mL
Staining Solution 5x [c]	15 mL
Additive Solution 1, 100x	1.5 mL
Additive Solution 2, 100x	1.5 mL
PBS 1x, pH 7,4 (powder) [d]	1 pouch

Order Info Kit Components: *Substrate (TBK0525-1) | Fixative Solution 10x Buffer (TBK0525-2) | Staining Solution 5x (TBK0525-3) | Additive Solution 1 (TBK0525-4) | Additive Solution 2 (TBK0525-5) | PBS pouch (TBB0600-1)*

Before its use

[a] **Substrate X-Gal Solution 20x**: Dissolve **20 mg Substrate** in 1 mL N,N, Dimethylformamide (CAS 68-12-2). Use a glass or a polypropylene container for X-GAL. Store at -20 °C.

[b] **Fixative Solution 1x**: Dilute 1 part of **Fixative Solution 10x** in **9 parts of Water**. Store at -20° C.

Fixative solution is toxic; it contains formaldehyde and glutaraldehyde and should therefore be handled with care to avoid inhalation or skin exposure.

[c] **Staining Solution 1x**: Dilute 2 part of **Staining Solution 5x** in 8 parts of Water. Store at 4°C.

[d] **PBS 1x:** Dissolve 1 pouch of PBS pH 7.4 in 1 L of sterile distilled water. Store at room temperature.

Staining Working Solution pH 6.0: In a polypropylene tube add,

	To prepare	
	1 mL	10 mL
Staining Solution 1x	930 µL	9.3 mL
Additive Solution 1, 100x	10 µL	100 µL
Additive Solution 2, 100x	10 µL	100 µL
Substrate Solution 20x	50 µL	500 µL

Storage

Store the kit protected from light at -20 °C.

PROTOCOL

This protocol is optimized for individual wells of a 6-well plate and can be adjusted as needed for other culture plate formats.

1. Seed 2–5×10^4 cells in either a 35-mm plate or 6-well plate, and culture for 2–3 days or more.
2. Remove culture medium and wash cells with **2 mL PBS 1x**.
3. For fixing cells or frozen tissue sections, add **1-2 mL Fixative Solution 1x** to submerge the cells.
4. Incubate for 10-15 minutes at room temperature.
5. Remove **Fixative Solution 1x** and wash the cells twice with **2 mL PBS 1x**.
6. Add **1 mL Staining Working Solution pH 6.0** to each well and cover the plate to prevent evaporation.

   The pH of the Staining Working Solution must be 6.0 to accurately detect SA-β-gal activity. A lower pH may lead to false positives, while a higher pH may cause false negatives. If necessary, adjust the pH using HCl or NaOH.
7. Incubate in the dark at 37°C, for 2 hours up to overnight.

   Do not incubate in a CO2 incubator. CO_2 can lower the pH and interfere with the development of the colorimetric reaction. Blue color is detectable in some cell lines within 2 hours, but staining is maximal at 12-16 hours.
8. After blue color is fully developed wash cells twice with **PBS 1x**.
9. Add one drop of mounting medium, and place cover glasses either on a 35-mm plate or 6-well plate. Observe the cells under a microscope.
10. Count the number of blue stained senescent cells and total cells. Calculate the percentage of senescent cells.