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ProductInformation

Senescence Cells Histochemical Staining Kit

Catalog Number **CS0030**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Cellular senescence is a progression of events, whereby cells move from an actively dividing to a non-dividing stage. The cellular senescence process is associated with aging. The decrease in cell division is virtually irreversible and complete. In conjunction with the loss of the ability to divide, changes occur in the morphology, shape, and physical appearance of the cells, and in their pattern of gene expression. At the end of the process cell death usually occurs, although the cells may remain viable for a long time.

The Senescence Cells Histochemical Staining Kit contains all the reagents required for identifying senescent cells using a rapid staining procedure. The assay is based on a histochemical stain for β-galactosidase activity at pH 6. Under these conditions, β-galactosidase activity is easily detectable in senescent cells, but undetectable in quiescent, immortal, or tumor cells.

Components

The kit is sufficient for 100 tests in 35 mm tissue culture plates.

Fixation Buffer 10× — 15 ml
(Catalog Number F1797)
Solution containing 20% formaldehyde, 2% glutaraldehyde, 70.4 mM Na_2HPO_4, 14.7 mM KH_2PO_4, 1.37 M NaCl, and 26.8 mM KCl

Reagent B — 1.5 ml
(Catalog Number R5272)
400 mM Potassium Ferricyanide

Reagent C — 1.5 ml
(Catalog Number R5147)
400 mM Potassium Ferrocyanide

X-gal Solution — 4 ml
(Catalog Number X3753)
40 mg/ml

Staining Solution 10× — 15 ml
(Catalog Number S5818)

Phosphate Buffered Saline (PBS) 10× — 60 ml
(Catalog Number P3621)

Reagents and Equipment Required but Not Provided

- 37 °C incubator
- Phase contrast or light microscope
- Cell culture plate
- 0.2 µm filter unit, Catalog Number F1387
- Glycerol, Catalog Number G5516 (optional)
- Parafilm®

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

The amounts of reagents detailed in the procedure are for 6 tests performed on 35 mm plates. For plates/wells of different sizes, calculate the volumes of the reagents required according to Appendix 1.

Before use, thaw all kit components and mix thoroughly until the solutions are homogenous.

Ultrapure water (17 MΩ·cm or equivalent) water should be used to prepare the working solutions for this kit.

X-gal Solution - Warm the X-gal Solution (Catalog Number X3753) at 37 °C for 1 hour. Warming this solution is very important to avoid formation of aggregates that could interfere with the visualization of the stained cells.

1× Fixation Buffer - Dilute the Fixation Buffer 10× (Catalog Number F1797) 10-fold with ultrapure water. Use of 0.2 μm filtered water is recommended. After preparation the 1× Fixation Buffer should be stored at –20 °C. Prepare 10 ml for 6 tests in a 35 mm plate or one 6 well plate.

1× PBS - Dilute the PBS 10× (Catalog Number P3621) 10-fold with 0.2 μm filtered ultrapure water. After preparation, the 1× PBS should be stored at 2–8 °C. Prepare 35 ml for 6 tests in a 35 mm plate or one 6 well plate. It is possible to prepare larger volumes and store the 1× PBS at 2–8 °C.

Staining Mixture - (Prepare just prior to use)
Mix the following for preparation of 10 ml of the Staining Mixture:

- 1 ml of Staining Solution 10× (Catalog Number S5818)
- 125 μl of Reagent B (Catalog Number R5272)
- 125 μl of Reagent C (Catalog Number R5147)
- 0.25 ml of X-gal Solution (Catalog Number X3753)
- 8.50 ml of ultrapure water

Filter the Staining Mixture using a 0.2 μm filter to ensure there are no aggregates in the solution.

Storage/Stability
The kit is shipped on dry ice and storage at –20 °C is recommended. After the initial thaw, store the PBS 10× and the Staining Solution at 2–8 °C.

Procedure

1. Aspirate the growth medium from the cells.
2. Wash the cells twice with 1 ml of 1× PBS per well/plate. Carefully remove the entire wash solution by aspiration, so the cells do not detach.
3. Add 1.5 ml per well of 1× Fixation Buffer and incubate the plate for 6–7 minutes at room temperature.
4. During the fixation process prepare the Staining Mixture as described in the Preparation Instructions.
5. Rinse the cells 3 times with 1 ml of 1× PBS per well/plate.
6. Add 1 ml of the Staining Mixture per well.
7. Incubate at 37 °C without CO_2 until the cells are stained blue (2 hours to overnight). Seal the plate with Parafilm to prevent it from drying out. The exact incubation time must be optimized.
   Note: The staining of senescent cells is pH dependent. Therefore, the cells **cannot** be incubated in a CO_2 enriched atmosphere during the staining step.
8. Observe the cells under a microscope. Count the blue-stained cells and the total number of cells. Calculate the percentage of cells expressing β-galactosidase (senescent cells).
9. After staining, if required, the Staining Mixture may be replaced with 1× PBS.
10. For long-term storage of the stained plate, aspirate the staining mixture, overlay the cells with a 70% glycerol solution, and store at 2–8 °C.

Appendix 1
Relative Volumes for Scale Up/Down of Staining Procedure

Plate	Well diameter (mm)	Growth area (cm^2)	Volumes relative to 35 mm plate
100 mm	100.00	78.50	8.26×
60 mm	60.00	30.00	3.15×
35 mm	35.00	9.50	1×
6 well	34.80	9.50	1×
12 well	22.10	3.80	0.4×
24 well	15.60	1.90	0.2×
96 well	6.40	0.32	0.034×

References

1. Dimri, I.G., et al., Proc. Natl. Acad. Sci. USA, **92**, 9363-9367 (1995).

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