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Senescence β-galactosidase staining assay in cultured cells

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Xin Xu[1], Yujuan Wang[1]

[1]Princess Margaret Cancer Centre

X Xin Xu
University Health Network

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We use this protocol and it's working

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Disclaimer

This protocol is provided for research use only and may require optimization depending on experimental conditions.

Abstract

This protocol describes senescence-associated β-galactosidase staining in cultured cells using a commercial staining kit to assess cellular senescence by light microscopy.

Materials

- Senescence β-Galactosidase Staining Kit (Cell Signaling Technology, 9860)

Safety warnings

- Staining time may vary depending on cell type and senescence level.
- Do not use a CO_2 incubator during the staining step, as CO_2 can alter pH.
- Include untreated and positive control samples for accurate interpretation.

Before start

- Perform all steps at room temperature unless otherwise indicated.
- Prepare thestaining solution fresh before use.
- Use a dry incubator **without** CO_2 for the staining reaction.
- Handle fixative and X-gal substrate according to institutional safety guidelines.

Cell preparation and positive control

1 Culture cells under experimental conditions until ready for senescence analysis.

2 (Optional positive control) Treat cells with 1 µM doxorubicin for 6 h prior to fixation to induce senescence-associated β-galactosidase activity.

Fixation

18m

3 Wash cells once with PBS. 1m

4 Fix cells with 1x Fixative Solution (provided in the Senescence β-Galactosidase Staining Kit, Cell Signaling Technology, 9860) for 10–15 min at room temperature. 15m

5 Rinse cells twice with PBS. 2m

β-galactosidase staining

6 Prepare fresh β-galactosidase staining solution (pH 6.0) containing X-gal substrate according to the manufacturer's instructions.

7 Add staining solution to completely cover cells.

8 Incubate cells at 37°C in a dry incubator without CO2 for 72 h.

Imaging and analysis

9 Examine cells under a light microscope.

10 Identify senescent cells by the presence of blue staining.

Expected result

11 Senescent cells exhibit clear blue β-galactosidase staining, whereas non-senescent cells remain unstained.