Oct 01, 2024

# SA-B-Gal Activity

DOI
**dx.doi.org/10.17504/protocols.io.3byl49n8ogo5/v1**

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**Protocol Citation:** Karyna Tarasova, Sinan Gültekin, iris.gerner Gerner, Florien Jenner 2024. SA-B-Gal Activity. **protocols.io** https://dx.doi.org/10.17504/protocols.io.3byl49n8ogo5/v1



**Protocol status:** Working
**We use this protocol and it's working**

**Created:** October 01, 2024

**Last Modified:** October 01, 2024

**Protocol Integer ID:** 108717

## Abstract

To measure cellular senescence

## Senescence associated SA-β-Galactosidase Activity

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1.1 The cells were washed twice with ice cold PBS and then incubated with 60µL of cell lysis buffer provided with the cellular senescence assay kit (Fluorometric format) (Cell Biolabs, USA) for 10 minutes at 4 ºC.

1.2 The whole cell lysates were centrifuged 15 minutes at 14000 g at 4 ºC. Total protein concentrations were determined by Qubit Protein Assay Kit (ThermoFisher, **Q33211**) using the Qubit 4 Fluorometer (Thermo Fisher Scientific, Singapore, shipped from Germany) according to the manufacturer's instructions.

1.3 Following protein quantification, the assay buffer (Cell Biolabs, Inc., CBA-231, San Diego, USA) was added 1:1 to the cell lysates and incubated for 3h in the dark at 37 ºC.

1.4 The reaction was stopped by adding 120 µL of the Stop solution (provided with the cellular senescence assay kit) per 30 µL of the reaction mixture.

1.5 Fluorescence was measured using a plate reader at 360 nm (Excitation) / 465 nm (Emission). SA-b-Gal activity was normalized to total protein concentrations.