ab65351 – Beta Galactosidase Staining Kit (Senescence)

For the rapid, sensitive and accurate measurement of Senescence in cultured cells tissue sections. For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab65351 (use http://www.abcam.cn/ab65351 for China, or http://www.abcam.co.jp/ab65351 for Japan)

Background:

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 Beta Galactosidase Staining Kit (Senescence) is designed to histochemically detect SA-β-Gal activity in cultured cells and tissue sections. The SA-β-Gal is present only in senescent cells and is not found in pre-senescent, quiescent or immortal cells.

Assay Summary:

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

Sample preparation

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Sample fixation (10 – 15 min at RT)

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Add Staining Solution Mix and incubate 1 hr -overnight at 37°C

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Observe sample under a microscope

QUICK ASSAY PROCEDURE

- Thaw Fixative Solution III, Staining Solution I and 100X Staining Supplement.
- Prepare X-gal solution.
- Prepare samples.
- Fix samples (10-15 min at RT).
- Wash samples with PBS.
- Prepare Staining Solution Mix and add 0.5 mL to each well.
- Incubate plate 1 hr - overnight at 37°C.
- Observe the samples under a microscope.

Precautions & Limitations:

Please read these instructions carefully prior to beginning the assay.
All kit components have been formulated and quality control tested to function successfully as a kit.

- Modifications to the kit components or procedures may result in loss of performance.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

Unsuitable for detection of senescent cells by Flow Cytometry. We recommend the fluorescent version of this kit, ab228562, for use in Flow Cytometry.

Storage and Stability:

Store kit at -20°C in the dark immediately upon receipt. Fixative Solution III, Staining Solution I and 100X Staining Supplement can be stored at 4°. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. Reconstituted components are stable for 1 month. Do not use kit or components if they have exceeded the expiry date.

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

Materials Supplied:

Item	Quantity	Storage Temperature (on receipt)	Storage temperature (reconstituted)
Fixative Solution III	125 mL	-20°C	-20°C or 4°C
X-Gal	1 vial	-20°C	-20°C
Staining Solution I*	125 mL	-20°C	-20°C or 4°C
100X Staining Supplement*	1.5 mL	-20°C	-20°C or 4°C

PLEASE NOTE: Fixative Solution III was previously labelled as 1X Fixative Solution, and Staining Solution I as 1X Staining Solution. The composition has not changed.

*** Note: If precipitation is observed, warm to 37°C in a water bath to resolubilize. If precipitation persists, centrifuge tube 5 min – 2000 rpm and use supernatant.**

Materials Required, Not Supplied:

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Light microscope
- 1 x PBS pH7.4
- DMSO or DMF (*N*,*N*-dimethylformamide)
- Sterile 12 well flat bottom tissue culture plate

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.
- Equilibrate reagents to room temperature before use.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.

Fixative Solution III, Staining Solution I and **100X Staining Supplement** are ready to use as supplied. Equilibrate to room temperature before use.

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 or glass container to make and store the X-gal**. Do not use polystyrene**.

Assay Procedure:

- Ensure X-gal solution and Staining Solution Mix is protected from light.
- Equilibrate all materials and prepared reagents to room temperature prior to use.
- The following protocol is designed for use with a 12-well plate. For larger plates, increase the volume proportionally (e.g., for 6-well plate, double the volumes in the protocol below).

1. Remove culture medium and wash cells once with 1 mL of 1X PBS per well.
2. Fix the cells or frozen tissue sections with 0.5 mL of Fixative Solution III per well for 10 - 15 min at room temperature.
3. **Staining Solution Mix:** While the cells are in the Fixative Solution III, prepare the Staining Solution Mix using a polypropylene plastic tube only (see table below). Prepare enough solution for the number of wells to be stained.
4. Wash the cells twice with 1 mL of 1X PBS per well.
5. 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: CO_2 levels found in general 37°C incubators will lower the pH of the Staining Solution I hereby affecting the color development. We suggest putting the plate inside a Ziplock® re-sealable bag to avoid any effect from the CO_2.
6. Observe the cells under a microscope for development of blue color (200X total magnification).

Component	Single Well (µL)	12 well Plate (µL)
Staining Solution I	470	5640
100X Staining Supplement	5	60
X-Gal solution	25	300

Technical Hints

For additional helpful hints and tips on using our assay kits please visit:
https://www.abcam.com/en-us/support/product-support

Technical Support

For technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)