Senescence Histochemical Staining Kit

Stain senescent cells/tissue using X-Gal to detect senescence-associated β-Gal (sa-β-Gal) activity.

(Cat. # 786-1068)

INTRODUCTION

Cellular Senescence is a chain of events frequently associated with ageing. The process leads dividing cells to completely exit the cell cycle and stop proliferating. Unlike quiescence it is an irreversible pathway that leads to several morphological and gene expression changes. Normal metabolism and cellular functions are drastically altered. The cell may remain in this condition for long periods of time although apoptosis may follow.

Senescence associated B-Galactosidase activity at pH 6 is one of the most frequently used biomarkers to detect senescent cells. This enzyme's activity at this specific pH is only observed in senescent cells due to the overexpression of endosomal β-Galactosidase. While there are several modern substrates that are more sensitive, X-Gal remains the most popular substrate to detect Sa- β -Gal. This is due to the simplicity of the assay as well as being one of the most established methods of detecting senescence and therefore less likely to raise questions among reviewers.

This kit was designed to be more convenient than other kits on the market. Our kit has been optimized to stain Sa- β -Gal specifically at pH 6 while remaining user friendly. Our high quality X-Gal substrate comes conveniently aliquoted in 4 vials to extend shelf-life and ensure high reproducibility from your first to last experiment. The kit provides 200ml assay volume, which can yield up to 400 assays using 24-well plates.

ITEMS SUPPLIED

Description	Size
Fixing Solution [10X]	20ml
Staining Solution [10X]	20ml
Staining Component A	20ml
Staining Component B	400µl
X-Gal Substrate	4x50mg
DMF	11ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store kit at -20˚C. After initial thaw, store "staining solution [10X]"at 4°C. X-Gal solution (20mg/ml DMF) is stable for around 1 month at -20°C protected from light. Discard if pink color develops. DMF can be stored at room temp. Allow reagents to warm to room temperature before use.

ADDITIONAL ITEMS NEEDED

- 1X PBS
- Sealing tape (cat# 786-422) or parafilm
- Ziplock type bags or 37°C incubator without CO_2
- Brightfield microscope

PRECAUTIONS

- Always use a polypropylene container or use the provided glass vial to dissolve or store X-Gal solution.

REAGENT PREPARATION

1. **Calculate the assay volume needed** for your particular experiment. This protocol is designed for 12-well culture plates using 0.5ml/well. Alternatively, assay volumes can be adjusted in proportion to culture area using the provided chart. *Always account for an extra well/plate than needed.*

Plate	Diameter (mm)	Area (cm^2)	Assay Volume (ml)	Wash Volume (ml)
100mm	100	78.5	8.25	10
60 mm	60	30	3.15	3.5
35 mm	35	9.5	1	1
6 well	34.8	9.5	1	1
12 well	22.1	3.8	0.5	0.5-1
24 well	15.6	1.9	0.25	0.3-0.5
96 well	6.4	0.32	0.04	0.2

2. Allow reagents to warm to room temperature before use. Mix well.
3. Dilute *Fixing solution [10X]* with molecular grade water.
4. Dissolve *X-GAL*: Add 2.5ml DMF per vial. Each vial contains enough substrate (50mg) to prepare 50ml staining solution.
   Once dissolved in DMF, the solution can be kept at -20°C for up to a month (in case less than 50ml assay is needed) Do not open vials if not needed right away.
5. **Prepare *Staining solution*** in a polypropylene tube or glass bottle. Use the table below to help calculate volumes.

Assay Vol.	Staining Solution [10X]	S. Component A [10X]	S. Component B [500X]	X-Gal soln. [20X]	H_2O
1ml	100µl	100µl	2µl	50µl	748µl
10ml	1ml	1ml	20µl	0.5ml	7.48ml
50ml	5ml	5ml	100µl	2.5ml	37.4ml
100ml	10ml	10ml	200µl	5ml	74.8ml
150ml	15ml	15ml	300µl	7.5ml	112.2ml
200ml	20ml	20ml	400µl	10ml	149.6ml

PROCEDURE

1. Aspirate media from the cells.

2. Wash cells with twice with 0.5ml PBS.

3. Add 0.5ml of 1X Fixing solution to each well. Incubate 5-10 minutes.

4. Wash cells 3 times with 0.5ml PBS.

5. Add 0.5ml staining solution to each well.

6. Seal plate with sealing tape or parafilm to prevent them from drying out.

7. Incubate at 37°C without carbon dioxide (CO_2) until the cells are stained blue. It may take from 2 to 14hrs until desired intensity is achieved. Pictures may be taken at different stages. *If no incubator without CO_2 enrichment is available, double ziplock bags may be used to isolate plates.*

8. Observe cells under a microscope for development of blue color. Estimate percentage of Sa β-Gal positive cells by estimating the percentage of positively stained cells. *Staining solution may be replaced with PBS to prevent further staining.*

9. *Cover cells with 70% glycerol solution for long-term storage. Store at 4°C.*

TROUBLESHOOTING

ISSUE	SOLUTION
Staining solution and staining supplement show precipitates.	Warm up the solution to solubilize precipitates.
Crystals formed in the wells/plates after incubation.	Problem due to solvent evaporation. Make sure plates are well sealed, especially when left overnight.
p53 was transduced and cells don't seem to be staining positive for Sa-B-Gal.	Especially with retroviral transductions cells need to be dividing for the overexpression to take place. Make sure the cells used don't express LT or E6/E7. Kit will detect cells once they enter senescence.

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