COS-0102

Senescence Detection Kit

(To stain 24-, 12-, 6-well plates, Store at 4°C)

Background Information:

Cellular Senescence is the phenomenon wherein cells stop dividing. It can be induced by both internal (oxidative stress, DNA damage, etc.) and external (toxins, UV light, etc.) factors Senescence is a state of arrested growth in which cells remain viable but stop dividing. Senescent cells display increase of cell size, senescence-associated expression of β-galactosidase, p53 and P16 activity, along with altered patterns of gene expression. AkrivisBio's Senescence Detection Kit is designed to detect β-Gal activity. β-Gal is present primarily in senescent cells but can give a false positive with cells that normally express β-gal such as mature macrophages.

Assay Principle:

1 – Cells are fixed to make them leaky
2 – Cells are stained overnight to allow for the blue color to develop and visualize under a microscope.

Assay Components:

Fixative Solution	125 ml	NM	COS-0102A
X-Gal (150 mg)	lyoph	Green	COS-0102B
Staining Solution	125 ml	WM	COS-0102C
Staining Supplement	1.5 ml	Red	COS-0102D

User Supplied Materials:

DMF
PBS

Storage and Handling:

Store the kit at -20°C. After dissolving X-gal, store solution at -20°C, protected from light.

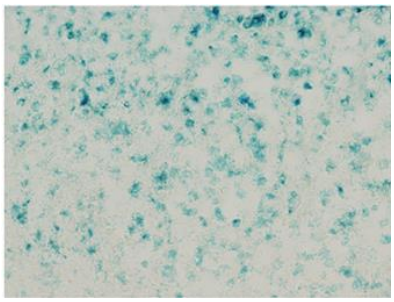
- The following protocol is designed for each well in a 12-well plate. If using a different plate, adjust volumes accordingly (e.g., for 6-well plate, double the volume, for a 24-well plate, halve the volume).
- Prepare PBS Solution (not provided). 3 ml per well.
- **X-gal Solution:** X-gal is hygroscopic. Allow it to come to room temperature for 30 minutes before opening the vial. Weigh out 20 mg X-gal to an amber or aluminum foil wrapped tube and add 1 ml N,N-dimethylformamide, to prepare a 20X stock solution. Unused X-gal solution can be stored at -20°C (protected from light) for 1-2 months. Always use a polypropylene or glass container with X-gal solutions. Do **not** use polystyrene.
- **Fixative Solution, Staining Solution and Staining Supplement** (100X) can be stored at 4°C.
- If precipitation occurs in the Staining Solution or Staining Supplement, warm the solution to 37°C to resolubilize the precipitates. If precipitation still persists, centrifuge the vial & use the supernatant.

Senescence Detection Protocol:

1. Remove culture medium from well by aspiration at the well edge and wash cells once with 1 ml of PBS.
2. Fix cells/with 0.5 ml of Fixative Solution for 10 - 15 min at room temperature. Prepare Stain while cells are being fixed. Prepare enough solution for the number of wells to be stained. For each well, prepare:

Staining Solution	470 µl
Staining Supplement	5 µl
X-gal in DMF	25 µl

3. Wash cells twice with 1 ml of PBS.
4. Add 0.5 ml of Staining Solution Mix to each well. Cover the plate. Incubate overnight at 37°C.
5. Observe the cells under a microscope for development of blue color (40 – 400 X).
6. For long-term storage of the stained plates, remove the Staining Solution and add 70% glycerol to the cells. Store at 4°C.



Typical result of X-gal staining.

Extracted from: Tominaga, T., Shimada, R., Okada, Y., Kawamata, T., & Kibayashi, K. (2019). Senescence-associated-β-galactosidase staining following traumatic brain injury in the mouse cerebrum. PLOS ONE, 14(3), e0213673. doi:10.1371/journal.pone.0213673

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