# Data sheet

## ROS Detection Assay kit

(DCFDA / $H_2$DCFDA)

**Cat No: CV0022 (5 x 96 assays)**

**Introduction:**

Senescence is one of the most fundamental aspects of cell behaviour and is thought to play a criti cal role in regulating cellular lifespan both in vitro and in vivo. The cell culture in vitro after a period o f rapid proliferation, cell division rate slows, and ultimately ceases altogether, with the cells bec oming unresponsive to mitogenic stimuli. This process is called Senescence. The senescence cells display a phenotype like increase of cell size, distinctive flat morphology, changes in gene expression and activity of senescence-associated β- galactosidase (SA-β-gal).
Senescence represents tumor suppressor mechanism for this reason cellular senescence has bec ome an increasingly target in the development of novel therapeutics. Senescence detection kit m easures activity of SA-β-Gal in cells cultures by hydrolysis of X-gal (5-Bromo-4- chloro-3-indolyl β-D-galactosidase), which results in the accumulation of a distinctive blue color in senescent cells. The SA-β-Gal is present only in senescent cells and is not found in pre-senescent, quiescent or immortal cells.

**Applications**

Detect SA-β-Galactosidase activity, a known characteristic of senescent cells, in cultured cell and tissue sections.

**Kit Contents**

| | Components | 100 assays |
|---|---|---|
| | X-Gal | 150 mg |
| | 1X PBS (Phosphate Buffered Saline) | 60 mL |
| | 10x Fixative Solution* | 15 mL |
| | 10X Staining Solution | 15 mL |
| | 100X Staining Solution Supplement A* | 1 x 1.5 mL |
| | 100x Staining Solution Supplement B* | 1 x 1.5 mL |

**Storage:**

The components can be stored at -20ºC and protected from light. Store reconstituted X-gal at -20 ºC. All components supplied are stable for 1 year.

**CAUTION**

* The fixative solution contains formaldehyde and glutaraldehyde, which are toxic and corrosive solutions. We ar personal protective clothing when handling solutions and use in a fume hood.

* Staining solution supplements (A and B) contains K4[Fe(CN)6] 3H2O and K3[Fe(CN)6], which are irritants for hu mans and dangerous for the environment. Wear personal protective clothing (e.g., nitrile or latex gloves, lab co at and goggles) when handling solution and discard in an appropriate manner.

**Reagent Preparation:**

1. **Prepare X-Gal solution:** Dissolve 20 mg in 1 ml DMF (N, N-dimethylformamide) to prepare a 20X stock solution. This solution must be freshly prepared or can be stored for one month at − 20 °C.

**CAUTION**

*Dimethylformamide is toxic and harmful. Wear personal protective clothing when handling solution. Use a fume hood. Always use a polypropylene container or glass to make and store the X-gal. Do not use polystyrene.*

2. **Prepare 1X Fixative Solution:** Prepare a 1X Fixative Solution by diluting the provided 10X stock 1:10 in water.
3. **Prepare 1X Staining Solution:** Prepare a 1X Staining Solution by diluting the provided 10X stock 1:10 in water.

If precipitation occurs in the solution, simply warming up the solution to solubilize the precipitates.

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# Assay Protocol

The following protocol is designed for each well in a 6-well plate (⌀ 35 mm) and may be modified accordingly to suit other culture plate sizes.

1. Remove culture medium and wash cells once with 2 mL of 1X PBS.

2. Fix the cells or frozen tissue sections: Add enough 1X fixative solution to submerge the cells (1–2 ml per well). Incubate for 5-10 minutes at room temperature.

3. While the cells are in the Fixative Solution, prepare the Staining Solution Mix using a polypropylene plastic tube only.

For each well, prepare:

- 1X Staining Solution: 930 µL
- 100X Staining Supplement A: 10 µL
- 100X Staining Supplement B: 10 µL
- 20 mg/ml X-gal in DMF: 50 µL

*The pH of Staining Solution Mix must be at 6.0. pH differences can affect staining: A low pH can result in false positives and high pH can result in false negatives. If necessary, use HCl or NaOH to lower or raise pH, respectively. The increased activity of SA-β-galactosidase is usually detected at pH 6, and constitutes the basis of the Senescent Cell Detection assay. Use a positive control in case there are no senescent cells in your conditions.*

4. Remove the **fixative solution** and wash the cells twice with 2 ml of **1X PBS**.

5. Add 1 ml of the **Staining Solution Mix** to each well. Cover the plate to prevent evaporation. Incubate plate in the dark at 37°C (2 hour – overnight incubation).

Do not incubate the cells in a CO2 incubator. CO2 levels found in general 37°C incubators will lower the pH of the staining solution thereby affecting the color development.

6. Observe the cells under a microscope for development of blue color. Count the blue stained cells and the total number of cells. Calculate the percentage of senescent cells (blue stained cells).

*Blue color is detectable in some cells within 2 h, but staining is maximal after 12–16 h. The exact incubation time must be optimized. Cells may be observed every 4 h during the first 12 h, and subsequently every 12 h.*

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