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Ready-to-Use Senescence β-Galactosidase Staining Kit

Cat #: KTA3031 Size: 100 T

	Ready-to-Use Senescence β-Galactosidase Staining Kit		
REF	**Cat #:** KTA3031	LOT	**Lot #:** Refer to product label
	Applicable samples: Cells and Frozen tissue sections		
	Storage: Stored at -20℃ for 12 months		

Assay Principle

Cells are the basic unit of biological structure and function, as well as the basic unit of biological aging. Cellular senescence is morphologically manifested as degeneration of cell structure, such as nuclear membrane depression, which eventually leads to nuclear membrane collapse, chromatin structure changes, and the number of hyperdiploid and abnormal polyploid cells increases; cell membrane fragility increases. Selective permeability decreases, and the types and numbers of membrane receptors and the sensitivity to ligands change; lipofuscin accumulates in cells, and many organelles and intracellular structures undergo degeneration. Cell senescence is physiologically manifested as functional decline and low metabolism, such as cell cycle arrest, loss of cell replication ability, weakened responsiveness to mitogenic stimulation, and changes in responsiveness to pro-apoptotic factors; intracellular enzyme active centers are oxidized, enzyme activity decreased, protein synthesis decreased, etc. The senescent cells can no longer replicate, but they still remain metabolically active and stain positive for senescence-associated beta-galactosidase activity, which is considered to be a biomarker of cellular senescence. The Senescence β-Galactosidase Staining Kit is a kit for staining and detecting senescent cells or tissues based on the up-regulation of senescence-related β-galactosidase activity level during aging. With X-Gal as the substrate, under the condition of pH 6.0, the senescent β-galactosidase catalyzes the formation of dark blue products, so that cells or tissues expressing β-galactosidase turn blue and can be observed under an optical microscope. This kit is suitable for the detection of senescence in cultured cells and tissue sections. It only stains senescent cells, and does not stain presenescent cells, quiescent cells, immortal cells or tumor cells.

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
	100 T	
Fixation Buffer	100 mL	-20℃
Reagent A	100 mL	-20℃
Reagent B	1.5 mL	-20℃
Reagent C	1.5 mL	-20℃
X-Gal solution	6 mL	-20℃, protected from light

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Note: This kit with 100 T specification is used for adherent cell detection. Taking a 96-well plate as an example, each well contains a 100 μL system, sufficient for 1000 detections; for a 48-well plate, each well contains a 250 μL system, sufficient for 400 detections; for a 24-well plate, each well contains a 500 μL system, sufficient for 200 detections; for a 12-well plate, each well contains a 1 mL system, sufficient for 100 detections; for a 6-well plate, each well contains a 2 mL system, sufficient for 50 detections. For suspension cells, taking a 1.5 mL EP tube as an example, each tube contains a 1 mL system, sufficient for 100 detections. For tissue sections and tissue blocks, the number of detectable samples depends on the size of the sample.

Materials Required but Not Supplied

- Optical microscope
- 37℃ incubator without CO_2
- Pipette and pipette tip
- Various glassware for preparing reagents and buffer solutions
- Deionized water, glycerin,70% ethanol
- Sealing film, Cell Culture Plate

Reagent Preparation

Reagent A: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.
Reagent B: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.
Reagent C: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.
X-Gal solution: Ready to use as supplied. Equilibrate to 37℃ before use. Store at -20℃, protected from light. **Warm the X-gal Solution at 37℃ for 1 hour is very important to avoid formation of aggregates that could interfere with the visualization of the stained cells.**
Staining Working Solution: Mix the reagents in the ratio of 930 μL Reagent A, 10 μL Reagent B, 10 μL Reagent C, 50 μL X-gal solution.

Assay Procedure

Note: Staining Working Solution is toxic and corrosive to the human body. Please be careful when handling and pay attention to effective protection. Avoid contact with human body or direct inhalation.
I For adherent cells:
1. Cultivate the cells directly on the cover glass of the 24-well plate and perform the required treatment.
2. Aspirate the cell culture solution and wash twice with 1×PBS.
3. Add 500 μL Fixation Buffer to each well and fix for 15 min at room temperature. For other types of culture plates, the amount of Fixation Buffer and subsequent solutions should be operated with reference to this ratio.
4. Aspirate the Fixation Buffer, and wash the cells 3 times with 1×PBS.
5. Aspirate 1×PBS and add 500 μL Staining Working Solution to each well.
6. Incubate overnight in a 37℃ incubator without CO_2 until the cells are stained blue.
Note: Seal the 24-well plate with parafilm to prevent the cells from drying out, and choose an appropriate staining time. Since cell senescence staining is related to pH, it is important to culture cells in a 37℃ incubator without CO_2.
7. Aspirate the Staining Working Solution, add 500 μL of 70% ethanol to each well, and wash for 10 minutes.
8. Remove 70% ethanol, wash twice with 1×PBS. Add 500 μL of 1×PBS.
9. Place the cells under an optical microscope for observation. Count the number of blue cells and the total number of cells, and calculate the percentage of cells expressing β-galactosidase (senescent cells). After staining, add 1×PBS; the cells can be stored at 4°C for several days or covered with a 70% glycerol solution for long-term storage at 4°C.
II For suspension cells:

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1. Centrifuge at 1,000 rpm for 5 min to collect the cells in a 1.5 mL centrifuge tube, and wash twice with 1×PBS.
2. Add 1 mL 1×Fixation Buffer to each tube, and fix for 15 min at room temperature. During fixation, you can slowly shake it on a shaker to prevent the cells from forming clumps.
3. After centrifugation at 800 g for 5 min, aspirate the Fixation Buffer, and wash the cells 3 times with 1×PBS.
4. Aspirate 1×PBS and add 1 mL Staining Working Solution to each tube.
5. Incubate overnight in a 37℃ incubator without CO_2 until the cells are stained blue.
Note: Seal the EP tube with plastic wrap to prevent cell desiccation, and choose an appropriate staining time. Since senescence staining is related to pH, cells cannot be cultured in an incubator containing CO_2.
6. Centrifuge at 800 g for 5 minutes, discard the Staining Working Solution, add 1 mL of 70% ethanol to each tube, and wash for 10 minutes.
7. Centrifuge at 800 g for 5 minutes, remove 70% ethanol, add 1×PBS, centrifuge at 800 g for 5 minutes and discard the supernatant, wash twice, and resuspend the cells in each tube with 200 μL of 1×PBS.
8. Take a portion of the stained cells and drop them onto a slide or into a 96-well plate, and observe under a standard optical microscope. Count the number of blue cells and the total number of cells, and calculate the percentage of cells expressing β-galactosidase (senescent cells). After staining, add 1×PBS; cells can be stored at 4°C for several days or covered with a 70% glycerol solution for long-term storage at 4°C.

III For tissue sections:

For paraffin sections, perform deparaffinization and hydration according to conventional methods. Follow the steps below directly for frozen sections.
1. For the prepared tissue section, add an appropriate volume of 1×Fixation Buffer to cover the tissue adequately, and fix for 15 min at room temperature.
2. Aspirate the Fixation Buffer and wash the cells 3 times with 1×PBS.
3. Aspirate 1×PBS and add an appropriate amount of Staining Working Solution.
Note: To prevent the liquid from drying out, it's recommended to stain it in a light-proof humid box, and it's best to immerse the entire section in the staining solution.
4. Incubate overnight in a 37°C incubator without CO_2 until the cells are stained blue.
5. Aspirate the Staining Working Solution, and soak the tissue in 70% ethanol for 10 minutes for washing.
6. Aspirate 70% ethanol, and soak the tissue in 1×PBS, washing it twice.
7. Place the tissue sections under an optical microscope for observation. Count the number of blue cells and the total number of cells, and calculate the percentage of cells expressing β-galactosidase (senescent cells). After staining, if immediate observation is not possible, cover the tissue sections with 70% glycerol solution and store at 4°C for long-term preservation.

Precautions

1. This kit only stains senescent cells, not presenescent cells, quiescent cells, immortal cells or tumor cells.
2. X-Gal solution will freeze when stored at -20℃ or 4℃, and it can be completely dissolved in a water bath at room temperature or 37℃ for 2-5 min with proper shaking.
3. Cell senescence β-galactosidase staining reaction depends on specific pH conditions, and the staining reaction cannot be performed in a CO_2 incubator. The higher concentration of CO_2 in the incubator will affect the pH value of the Staining Working Solution and cause staining failure. For the porous plate, it can be sealed with parafilm or plastic wrap to prevent evaporation.
4. If there is precipitation after the reagent is thawed or before use, it must be ensured that all the precipitation is dissolved before use. When Reagent C is just taken out of the kit, there may be a small amount of precipitation at the bottom of the tube, which is a normal phenomenon. After mixing thoroughly, the precipitation will be completely dissolved. Make sure to use it after all of it is dissolved. When preparing the Staining Working Solution, a small amount of flocculent precipitate may also appear, which will be completely dissolved after shaking and mixing, and it must be used before it is completely dissolved.

Recommended Products

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Catalog No.	Product Name
KTA2030	EdU Cell Proliferation Image/Flow Cytometry Assay Kit (Green Fluorescence)
KTA2031	EdU Cell Proliferation Image/Flow Cytometry Assay Kit (Orange Fluorescence)
KTA2010	TUNEL Apoptosis Detection Kit (Green Fluorescence)
KTA2011	TUNEL Apoptosis Detection Kit (Orange Fluorescence)

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