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Technical Datasheet

EZdetect™ Cell Senescence Detection Kit

Product Code: CCK063

1. Introduction

Cell senescence is a physiological process in which the cell population reaches the end of its proliferative life span. In culture, normal cells undergo a finite number of population doublings after which both DNA and cellular division cease, but metabolic activity and cellular viability may be maintained for an extended period of time. The loss of proliferative potential in these *in vitro* systems is termed cellular senescence.

The phenomenon of cellular senescence has gained immense importance due to its emerging physiological role in tumor suppression and tissue wound repair. In recent oncology research, it has been demonstrated that exposure to ionizing radiations and cancer therapeutic drugs results in senescence-like growth arrest in cultures if variety of solid tumor-derived human cell lines, thus making cellular senescence a valuable tool to determine efficacy of anti-cancer drugs *in vitro*.

2. About the Assay

EZdetect™ Cell Senescence Detection Kit is designed for detection of cell senescence in culture. This kit is based on the qualitative detection of senescent cells that exhibit increased activity of acidic β-galactosidase, termed senescence-associated β-galactosidase (SA-β-gal). SA-β-gal is the most widely accepted marker of senescent cells. The activity derives from residual lysosomal β-galactosidase activity at the suboptimal pH 6.0 and reflects the increased lysosomal content of senescent cells.

3. Kit contents

The reagents supplied in the kit are sufficient for 100 assays in 35mm cell culture dish.

Code	Description	Quantity	Storage
CCK063(A)	Staining solution A 10X	15ml	-20°C
CCK063(B)	Staining solution B	1.5ml	-20°C
CCK063(C)	Staining solution C	1.5ml	-20°C
CCK063 (D)	X-gal solution	4ml	-20°C
CCK063(E)	Fixing solution 10X	15ml	-20°C
CCK063(F)	Dulbecco's Phosphate Buffered Saline 10X	60ml	Room temperature

4. Materials required but not provided in the kit

- Adjustable pipettes and a repeat pipettor
- Incubator at 37°C
- Inverted microscope
- Parafilm
- Glycerol
- Cell culture grade water
- Polypropylene tubes

5. Directions for use

Users are advised to review entire procedure before starting the assay

5.1. Preparation of reagents

5.1.1. 1X Fixing solution

Note: Prepare exact required quantity of fixing solution. Do not prepare extra.

1. Dilute 10X fixing solution CCK063(E) with sterile cell culture grade water in 1:9 ratio to obtain 1X fixing solution.

2. Store 1X fixing solution at -20ºC after use.

5.1.2. 1X Dulbecco's Phosphate Buffered Saline

1. Dilute 10X Dulbecco's Phosphate Buffered Saline CCK063(F) with sterile cell culture grade water in 1:9 ratio to obtain 1X Dulbecco's Phosphate Buffered Saline.
2. Store it at room temperature after use.

5.1.3. X-gal solution

1. Thaw X-gal solution CCK063(D) at room temperature.
2. Warm the thawed solution at 37ºC water bath for 1 hour.
   Note: Warming of X-gal solution avoids formation of aggregates that could interfere with the visualization of stained cells.

5.1.4. Staining mixture

1. Thaw staining solution A 10X CCK063(A) at room temperature.
2. Prepare staining mixture in polypropylene tube as given in table just prior to use.

Table 1: Composition of staining mixture

Component	Volume (ml)	
	For 10ml	For 20ml
Staining solution A 10X CCK063(A)	1ml	2ml
Staining solution B CCK063(B)	125µl	250µl
Staining solution C CCK063(C)	125µl	250µl
X-gal solution CCK063(D)	250µl	500µl
Cell culture grade water	8.5ml	17ml

3. Filter the staining mixture through 0.22µ syringe filter to ensure that the solution is free of any aggregates.

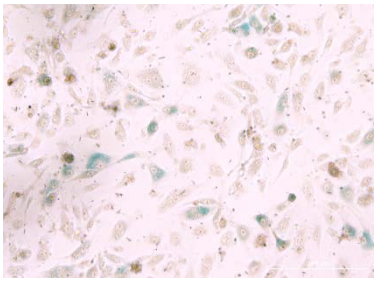
5.1.5. Staining procedure

Note: Volumes of reagents mentioned in the procedure are for 35mm cell culture dish. Refer table 2 for volumes of reagents to be used for other culture vessels.

1. Aspirate the growth medium from cells.
2. Add 1ml 1X DPBS to each the dish and swirl gently to remove traces of medium.
3. Discard DPBS by aspiration and repeat washing step once again.
4. Add 1ml 1X fixing solution to the dish and incubate the plate at room temperature for 8 – 10 minutes.
   Note: During fixation time, prepare staining mixture.
5. Discard fixing solution after incubation by aspiration.
6. Add 1ml 1X DPBS and swirl gently to remove traces of fixing solution.
7. Repeat washing twice.
8. Add 1ml staining mixture to the dish and incubate the plate at 37ºC without CO_2 for 2 hours or overnight.
   Note: Do not incubate the cells in CO_2 incubator for staining because SA-β-gal activity is pH dependent.
9. Observe the cells under a microscope at regular intervals and optimize the exact incubation time.
10. Count the blue stained cells and total number of cells. Calculate the percentage of senescent cells.
11. If required, remove the staining solution after staining and replace it with same volume of 1X DPBS.
12. For long term storage of stained plates, aspirate the staining mixture and overlay the cells with 70% glycerol solution and store at 2 – 8ºC.

Table 2: Reagent volumes for various culture vessels

Culture vessel	Reagent volumes	No. of tests in one kit
100mm cell culture dish	8ml	12
60mm cell culture dish	3ml	30
35mm cell culture dish	1ml	100
6 well plate	1ml	100
12 well plate	500µl	200
24 well plate	250µl	400
48 well plate	200µl	500
96 well plate	100µl	1000



Senescent HUVEC cells

6. Storage and shelf life

Store 10X DPBS and 1X DPBS at room temperature.
Store other components at -20ºC.
Use before expiry date mentioned on the product label.

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