ELK Biotechnology

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DNA Damage Assay kit

Catalog No.	Specification	Storage
BC149-1	20 T	4 C°
BC149-2	50 T	4 C°

Introduction

DNA is prone to damage under the attack of free radicals (such as ·OH), that is, deoxypentose is broken, the diphosphate bond is broken, and the base is broken or shed, which can further produce single-strand break or double-strand fracture. Fix the cells in low-melting agarose, apply a small amount of cells on a glass slide, destroy the cell membrane with an alkaline high-salt solution, and untwist the DNA molecules with the alkaline solution. Place the slide in the electrophoresis solution, and under the action of the electric field, the DNA molecules move towards the anode. If the DNA damage is severe and there are many fragments, the electrophoresis speed is fast. Undamaged DNA macromolecules stay in place due to the barrier of the cell membrane. With EB, PI staining or silver staining, it can be observed that cells with damaged DNA resemble comets, for qualitative analysis. Related software can also be used for quantitative analysis.

Kit Components

Components	20 T	50 T	Storage
Lysis Bufffer	100 mL	250 ml	RT
DMSO	10 mL	25 ml	RT
4mM Tris-Hcl	40ml	100ml	RT
Low melting point agarose LMA	4ml	10ml	4℃
Normal melting point agarose NMA	20mg	50mg	RT
Propidium lodide (PI)	400 μL	1 ml	4℃
Two-hole slides	10pieces	25pieces	RT

Required equipment and reagents

Low speed centrifuge, horizontal electrophoresis instrument, fluorescence microscope, constant temperature water bath at 37℃ and 45℃,, coverslips, plates,

Micropipette, 1.5 ml Microube, 0.4 mmol/L Tris-HCl (pH7.5) buffer, PBS

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Note:

PI is toxic, wear gloves when handling.

Operation steps:

1. The cells were washed once with ice-cold PBS, collected by centrifugation, and resuspended with PBS to make the density $1x10^6$ cells/mL;

2. Spreading gel: the following concentrations of agarose gel are prepared with PBS, the preparation of the first layer of gel: the matte side of the slide glass is preheated at 45℃, preheated at 45℃ 100 μL 0.5%
Normal melting point agarose (NMA) is spread on the slide, covered with a clean cover slip, and then placed at 4℃ for 10min to make NMA solidification.
Preparation of layer 2 gel: 10 μL of cells (about 10^4 cells) and 75 μL of 0.7% low melting point agarose LMA
(Heave at 37°C in a water bath for at least 20 minutes to completely dissolve) Mix well. Then, gently remove the cover slip
Quickly drop the LMA containing cells onto the first layer of agarose, immediately cover with another clean cover slip, and set at 4°C for 10 min to solidify the second layer of LMA.
Preparation of the third layer of gel: After the second layer of LMA is solidified, carefully remove the cover glass at room temperature and add preheated 37°C dropwise
75 μL of 0.7% low-melting-point agarose LMA, such as a cover glass and solidify at 4 ℃ (the third layer covers 0.5mm around the second layer, increase the gelation time to 30min, high humidity environment).

3. Remove the coverslip from the cell lysis, place the slide in a flat dish, pour into the pre-cooled Lysis Bufffer (add 1 mL of DMSO every 9 mL before use), lyse at 4℃ for 1~2 h, remove the slide and rinse with PBS .

4. DNA alkaline unwinding Place the slides in a horizontal electrophoresis tank. Pour into the newly prepared alkaline electrophoresis buffer (user-supplied 1 mmol/L EDTA, 300 mmol/L NaOH), cover approximately 0.25 cm of the surface of the overloaded slide glass, and leave it at room temperature for 20~60 min to keep the DNA under alkaline conditions Helix unwinding and

generation of alkali volatile segments make DNA strands easily migrate in the electric field.

5. Single cell electrophoresis At a voltage of 25V and an electrophoresis of 20~30 min, the voltage and current can be adjusted by changing the level of the buffer.

6. Neutralization and staining After electrophoresis, place the slides in a dish. Add 0.4mmol/L Tris-HCl (ph7.5) buffer, immerse the slides, neutralize three times at 4℃ for 10min each time, discard Tris-HCl buffer, add 20 μL of PI staining solution per slide or EB staining solution, covered with a coverslip and stained for 10 min in the dark.

7. Observe, take pictures and analyze fluorescence microscope excitation light of 515~560 nm wavelength, PI-stained DNA image is red, which can clearly observe nuclear DNA and migrating DNA (ie Hui Star tail).

100 cells were randomly selected from each sample to determine the diameter of nuclear DNA and the length of DNA migration, which can be analyzed with corresponding software.

DNA damage is divided into 5 levels according to the proportion of comet tail DNA to the total DNA:

Level 0: <5% without damage;

Level 1 5 ~ 20% mild damage;

Level 2 20 ~ 40% moderate damage;

Level 3 40 ~ 95% high damage;

Level 4> 95% severe damage.