Comet Assay Kit (3-Well Slides)

Cat #: KTA3040 Size: 15 T/75 T

	Comet Assay Kit (3-Well Slides)		
REF	**Cat #:** KTA3040	LOT	**Lot #:** Refer to product label
	Applicable samples: Tissue, Cells		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Comet Assay Kit (3-Well Slides) is a single cell gel electrophoresis assay (SCGE), rapid and sensitive test for detecting DNA damage in cells. The principle is that after organs or tissues have been suffered from (such as radiation, heavy metals, etc.), single strand or double strand DNA was broken in the cells, the cell is mixed with melted agarose and added on a slide, then the cell membrane is disrupted with lysis solution, the DNA is uncoiled with alkaline solution. Finally, the samples were electrophoresed, under the action of an electric field, the macromolecular DNA of normal cells migrates for a shorter distance, intact DNA remains in the range of the nucleus, broken pieces of DNA migrate out of the nucleus, stained with PI dye, observed under a fluorescence microscope, intact DNA forms a circular or slightly tailed pattern; DNA fragmentation fragments form comet-like pattern. According to the pH value of the electrophoresis buffer, it can be divided into neutral comet assay (pH=8.4) and alkaline comet assay (pH>13). Neutral comet assay was mainly used to detect the broken damage of DNA double strand, and alkaline comet assay has higher sensitivity and can be used to detect less single strand and double strand broken damage.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	15 T	75 T	
Comet Slides (3-Well)	5	25	4℃
10×Lysis Solution	15 mL	75 mL	4℃
EDTA (500 mM)	25 mL	125 mL	4℃
Agarose (Low Melting Point)	15 mL	15 mL×5	4℃
Propidium Iodide (PI) (50×)	20 μL	100 μL	4℃, protected from light
40 mM Tris-HCl (pH 7.5)	15 mL	75 mL	4℃

Materials Required but Not Supplied

- Fluorescence microscope

- Centrifuge, 37℃ and boiling water bath, horizontal electrophoresis chamber, precision pipettes, disposable pipette tips
- Deionized water, NaCl powder, NaOH pellets, boric acid, EDTA (disodium salt), DMSO (optional)
- 10 M NaOH for pH adjustment, 70% ethanol
- PBS (without $Mg^{2+}$and Ca^{2+}, pH 7.4)

Reagent Preparation

Comet Slides (3-well): Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

10×Lysis Solution: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

EDTA (500 mM): Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Agarose (Low Melting Point): The bottle of Agarose (Low Melting Point) was heated in a water bath at 90-95℃ for 20 min until the Agarose liquefied. The bottles were transferred to a 37℃ water bath for 20 min to maintain the liquid state. The rest was aliquoted and stored at 4℃ for 6 months. The color of the Agarose is yellow, which is caused by high temperature autoclave sterilization and will not affect the use.

Propidium Iodide (PI) (50×): Prepared freshly before use and diluted 50 fold with PBS to a concentration of 1×PI dye, stored at 4℃ and protected from light.

40 mM Tris-HCl (pH 7.5) : Prepared before use and diluted 100 fold with PBS to a concentration of 0.4 mM Tris-HCl (pH 7.5) and stored at 4℃.

1×Lysis Buffer: Prepare 100 mL 1×Lysis Buffer, formulated as follows:

Reagent	Quantity
NaCl	14.6 g
EDTA (500 mM)	20 mL
10×Lysis Solution	10 mL
DMSO	10 mL (optional for heme containing samples)
Deionized Water	Adjust volume to 90 mL
Mix well before use, adjust pH to 10 with 10 M NaOH, and adjust volume to 100 mL with deionized water. Cooled at 4℃ for at least 20 min before use.	

Note: Reagents are freshly prepared; For heme containing samples, such as blood cells and tissues, DMSO can be added at a final concentration of 10%. Cool 1×Lysis Buffer at 4℃ for at least 20 min before use. The 1×Lysis Buffer will look cloudy at room temperature, but it will become clear at 4℃ and the pH will remain around 10.

Alkaline Solution: Prepare 100 mL Alkaline Solution, formulated as follows:

Reagent	Quantity
NaOH	1.2 g
EDTA (500 mM)	0.2 mL
Deionized Water	Adjust volume to 100 mL

Note: mix well before use. Alkaline Solution is freshly prepared, and cooled at 4℃ for at least 20 min before use.

Choice of electrophoresis solution:

Choose the appropriate electrophoresis solution based on the desired running conditions and detection sensitivity. TBE is preferred for the analysis of apoptosis and enables data analysis using tail length rather than tail moment. TBE electrophoresis detects both single-stranded and double-stranded DNA breaks and may detect a few AP sites. Alkaline electrophoresis is more sensitive and can detect smaller amounts of DNA damage. Alkaline electrophoresis can detect single-stranded and double-stranded DNA breaks, the majority of AP sites, and alkali labile DNA adducts.

1. TBE Electrophoresis Solution

Reagent	Quantity
Tris Base	10.8 g
Boric Acid	5.5 g
EDTA (disodium salt)	0.93 g

Deionized Water	Adjust volume to 1 L

Note: mix well before use. TBE Electrophoresis Solution is freshly prepared, and cooled at 4℃ for at least 20 min before use.

2. Alkaline Electrophoresis Solution (300 mM NaOH, 1 mM EDTA, pH>13)

Reagent	Quantity
NaOH	12 g
EDTA (500 mM)	2 mL
Deionized Water	Adjust volume to 1 L

Note: mix well before use. Alkaline Electrophoresis Solution is freshly prepared, and cooled at 4℃ for at least 20 min before use.

Assay Procedure

A. Preparation of samples

1. Prepare 1×Lysis Buffer, Alkaline Solution, and Electrophoresis Solution (see Reagent Preparation) prior to performing the assay. Cool all solutions to 4℃ before use.

2. Heat Agarose (Low Melting Point) to 90-95℃ in a water bath for 20 min until Agarose liquefies, then transfer the bottle to a water bath set to 37℃ for 20 min for cooled and storage.

3. Comet Slide was pre-warmed at 37℃. Add 100 µL Agarose per well onto the Comet Slide to create a base layer and ensure complete well coverage. Maintaining the Comet Slide horizontally, transfer the Comet Slide to 4℃ for 15 min.

4. Prepare cell samples, as follows:

Suspension Cells: Centrifuge cells at 1,000 g for 5 min and discard supernatant. Wash cells once with ice-cold PBS, centrifuge, and discard the supernatant. Finally, resuspend the cells at 1-5×10^5 cells/mL in ice-cold PBS.

Adherent Cells: Gently remove the cells from the dish by cell scraping. Transfer cell suspension to a centrifuge tube and centrifuge at 1,000 g for 5 min, discarding the supernatant. Wash cells once with ice-cold PBS, centrifuge, and discard the supernatant. Finally, resuspend the cells at 1-5×10^5 cells/mL in ice-cold PBS.

Tissue Preparation: Using dissection scissors, cut a small piece of tissue into pieces and add 1-2 mL ice-cold PBS containing 20 mM EDTA , Keep the tissue/cell suspension for 5 min without moving and transfer the supernatant to a centrifuge tube avoiding transferring debris. Centrifuge at 1,000 g for 5 min, and discard the supernatant. Finally, resuspend the cells at 1-5×10^5 cells/mL in ice-cold PBS.

5. Combine cell samples with Agarose at 1:10 ratio (V/V), mix well by pipetting, and immediately transfer 75 µL/well onto the top of the Agarose base layer for complete coverage without disturbing the base layer.

6. Maintaining the Comet Slide horizontally, transfer the Comet Slide to dark room for 15 min at 4℃.

7. Transfer the Comet Slide horizontally to pre-chilled 1×Lysis Buffer (approximately 25 mL/Comet Slide) in a container for 30-60 min at 4℃ in the dark.

8. Aspirate the 1×Lysis Buffer from the container carefully, and replaced with pre-chilled Alkaline Solution (approximately 25 mL/Comet Slide). Immerse the Comet Slide in the solution for 30 min at 4℃ in the dark.

Note: 1. Prior to formal experimentation, pre-experiments are required to determine the optimal number of cells and lysis time. 2. For multiple samples, keep cell samples at 37℃ with agarose mix to avoid gelation. 3. To avoid damage to cell samples by UV, perform the assay under low/dark light conditions.

B. Electrophoresis

Note: choose an appropriate electrophoresis solution based on the desired running conditions and detection sensitivity.

1. TBE electrophoresis:

(1) Aspirate the Alkaline Solution from the container and replace with pre-chilled TBE Electrophoresis Solution. Immerse the Comet Slide for 5 min, and then repeat this step once.

(2) Maintaining the Comet Slide horizontally, carefully transfer the Comet Slide to a horizontal electrophoresis chamber. Fill the chamber with cold TBE Electrophoresis Solution until the buffer level covers the Comet Slide.

(3) Apply voltage to the chamber for 10-15 min at 1 volt/cm (e.g. if the chamber electrodes are 35 cm apart, you would then apply 35 volts to the Comet Slide), and the voltage, current can be adjusted by varying the level of the electrophoretic liquid surface.

(4) Maintaining the Comet Slide horizontally, carefully transfer the Comet Slide from the electrophoresis chamber to a clean, small basin/container containing pre-chilled deionized water (approximately 25 mL/Comet Slide). Immerse the Comet Slide for 2 min, aspirate, and then repeat this step twice.

(5) Remove deionized water, add pre-chilled 70% ethanol (approximately 25 mL/Comet Slide), and immerse for 5 min.

(6) Maintaining the Comet Slide horizontally, remove the Comet Slide from the 70% ethanol and allow to dry by air.

(7) After Agarose and Comet Slide were completely dried, 50 μL/well configured 1×PI dye and stained for 10 min in the dark at room temperature.

(8) After PBS wash once, the Comet Slide can be analysed and recorded using fluorescence microscope.

2. Alkaline electrophoresis:

(1) Maintaining the Comet Slide horizontally, carefully transfer the Comet Slide from the Alkaline Solution to a horizontal electrophoresis chamber. Fill the chamber with cold Alkaline Electrophoresis Solution until the buffer level covers the Comet Slide.

(2) Apply voltage to the chamber for 15-30 min at 1 volt/cm (e.g. if the chamber electrodes are 35 cm apart, you would then apply 35 volts to the Comet Slide), and the voltage, current can be adjusted by varying the level of the electrophoretic liquid surface.

(3) After electrophoresis, Comet Slide were horizontally transferred to a container containing pre-cooled 0.4 mM Tris-HCl (pH 7.5) buffer (approximately 25 mL/Comet Slide) and neutralized 3 times for 10 min each at 4℃.

(4) The Tris-HCl buffer was discarded and 50 μL/well configured 1×PI dye and stained for 10 min in the dark at room temperature.

(5) After PBS wash once, the Comet Slide can be analysed and recorded using fluorescence microscope.

Note: after alkaline electrophoresis, take pictures as soon as possible, and if not, immerse the comet slide in PBS, protected from wetting at 4℃.

Data Analysis

The DNA damage is quantified by measuring the displacement between the genetic material of the nucleus ('comet head') and the resulting 'tail'. Tail Moment and Tail DNA% are the two most common parameters to analyze Comet assay results. At least 50-100 cells should be analyzed per sample. The Tail Moment has been suggested to be an appropriate index of induced DNA damage in considering both the migration of the genetic material as well as the relative amount of DNA in the tail.

Tail DNA%=Tail DNA Intensity/Cell DNA Intensity×100

Tail Moment can be measured using one of the following methods:

(a) Olive Tail Moment=Tail DNA%×Tail Moment Length (measured from the center of the head to the center of the tail, see Figure 1)

(b) Extent Tail Moment=Tail DNA%×Tail Length(see Figure 1)

Comet Assay results analysis software is available through Opencomet, CASPlab and Comet Assay IV (Perceptive Instruments), among others.

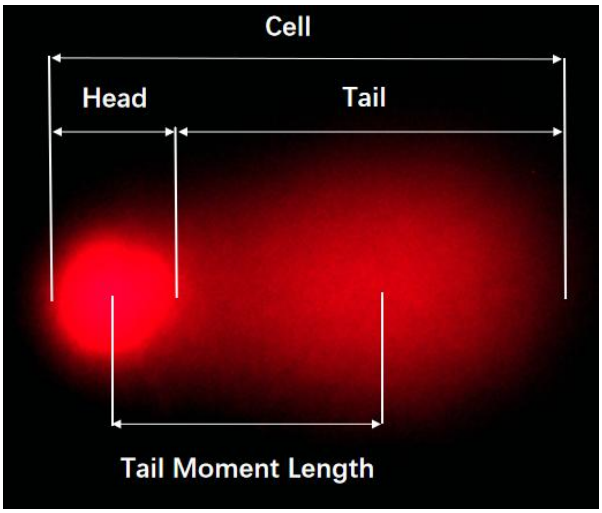

Figure 1. DNA damage in Comet Assay.

Qualitative method: DNA damage was classified into five levels according to the proportion of Tail DNA%

Grade	Percent of DNA Damage	Damage Degree
0	< 5%	no damage
1	5-20%	mild impairment
2	20-40%	moderate impairment
3	40-95%	high grade injury
4	> 95%	severe injury

Example:

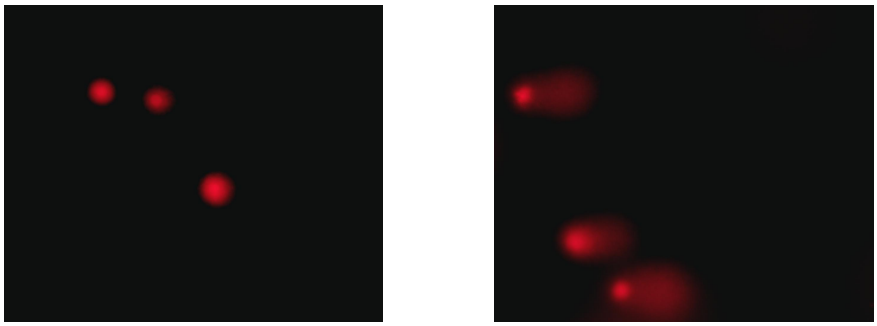

Figure 2. Jurkat cells were untreated (left) or treated (right) with 20 μM etoposide for 4 hours before performing Comet Assay (alkaline electrophoresis conditions, 21 V for 20 min).

Recommended Products

Catalog No.	Product Name
KTA0002	Annexin V-AbFluor™ 488/PI Apoptosis Detection kit
KTA2010	One-step TUNEL Apoptosis Assay Kit (Green Fluorescence)
KTA2011	One-step TUNEL Apoptosis Assay Kit (Orange Fluorescence)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.