MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Comet Assay Kit

Product Number: CAK041

Shipping and Storage

Store at 4ºC, valid for one year. The Propidium Ioide Solution must be stored in a dark place. DMSO, Normal Melting Point Agarose, and Low Melting Point Agarose can also be stored at room temperature.

Components

Component	CAK041S 20rxns	CAK041M 100rxns
Lysis Buffer	200ml	500ml×2
DMSO	20ml	100ml
Normal Melting Point Agarose	100mg	500mg
Low Melting Point Agarose	70mg	350mg
Propidium Iodide Solution	400μl	2ml

Description

Comet Assay Kit, also known as DNA Damage Comet Assay Kit, is a simple, fast, and efficient kit for detecting single-cell DNA damage through electrophoresis. The Comet Electrophoresis Assay, also known as Single Cell Gel Electrophoresis Assay (SCGE), is a rapid experimental method for detecting DNA damage observed in single cells. It is called Comet Electrophoresis or Comet Assay because the image of DNA damaged cells observed during electrophoresis resembles a comet.

Common DNA damages include base modifications, intra - and inter strand cross-linking, single strand breaks (SSBs), and double strand breaks (DSBs), among which DSBs are considered the most severe DNA damage. The physical or chemical factors that cause DNA to produce DSBs include ultraviolet radiation, ionizing radiation (X-rays, Y-rays, etc.), genotoxic chemicals, and chemotherapy drugs. The ineffective or incorrect repair of DSBs can cause genomic disorders, ultimately leading to the occurrence of tumors and other related diseases.

According to the principle of DNA damage, DNA damage detection can be divided into three categories: based on changes in the physical and chemical properties of damaged DNA, such as comet experiments; Based on molecular hybridization, such as fluorescence in site hybridization (FISH); Detecting DNA damage based on products formed after DNA damage, such as detecting phosphorylated H2AX, exposed 3 '- OH (TUNEL detection), and 8-OHdG. The first two methods are based on fluorescence labeling to directly observe DNA damage, while detecting DNA damage based on the products formed after DNA damage indirectly reflects the degree of DNA damage through markers. This comet electrophoresis kit is based on fluorescence labeling for direct detection of DNA damage. It was originally invented by Östling and Johansson in 1984 and improved by Singh et al. in 1988. It has since become a standard experimental technique for evaluating DNA damage and repair, as well as genetic toxicity testing.

Comet electrophoresis is a commonly used method for detecting DNA damage in eukaryotic cells, and its detection principle is as follows. The low melting point agarose gel has the characteristics of maintaining the solution state at 37℃, which can maintain the original state of cells without causing additional DNA damage, and avoid cell damage caused by the normal melting point agarose melting at a higher temperature. Therefore, the cells were embedded on a slide containing low melting point agarose gel. After the cells were lysed, the migration rate of macromolecular DNA in the normal cell nucleus was basically the same under the action of electric field due to its integrity, and the DNA fluorescence staining could still maintain the shape of the nucleus or have an unusually slight tailing; If DNA undergoes single or double strand breaks, under the action of an electric field, DNA with different molecular weights exhibits different migration rates. Large molecule DNA has a lower migration rate, while small molecule DNA fragments have a faster migration rate. After fluorescence staining, large molecule DNA still presents a nucleus like shape, resembling a Comet head, while small molecule DNA shows a tail shape that escapes from the nucleus, resembling a Comet tail. The more severe the DNA damage, the more small molecular DNA fragments there are, and the smaller the fragments, the faster the migration rate during

electrophoresis, and the longer the comet like tail. Therefore, the degree of DNA damage to individual cells can be quantitatively determined by measuring the optical density or length of the comet tail. The effect of using this reagent kit to detect DNA damage in HeLa cells is shown in Figure 1

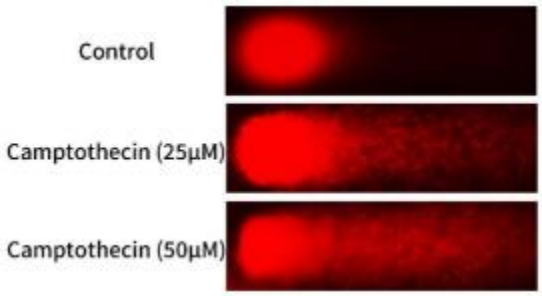

Figure 1. Effect of Comet Assay Kit (CAK041) in detecting DNA damage in HeLa cells.

Under normal culture conditions, there is almost no comet like tail in HeLa cells (Control), indicating that the DNA is very intact; After 1 hour of induction with 25μM or 50μM Camptothecin, some nuclei showed obvious comet tails, indicating varying degrees of DNA damage. The actual dyeing effect may vary due to different experimental conditions, testing instruments, etc. The effect in the figure is for reference only.

Application

According to the differences in lysis solutions, comet electrophoresis can be divided into neutral comet electrophoresis and alkaline comet electrophoresis. Neutral comet electrophoresis, mainly used for detecting DNA double strand breaks; Alkaline comet electrophoresis has higher sensitivity and can detect fewer single and double strand breaks, alkaline instability sites of DNA, and DNA strand breaks caused by incomplete excision repair. This reagent kit uses the commonly used alkaline comet electrophoresis method.

Note

1. Lysis Buffer must be completely dissolved before use. If there is precipitation, it can be fully dissolved in a 37℃ water bath.
2. Starting from the cell lysis step, all reagents should be pre cooled at 4℃ before use in cells, and maintained at a low temperature of 4℃ and in a dark place during lysis, dissociation, electrophoresis, and neutralization to prevent inducing additional DNA damage. Pay special attention to avoiding damage to DNA caused by ultraviolet rays in sunlight, fluorescent lamps, etc.
3. PBS without calcium or magnesium ions needs to be used
4. The positive control for DNA damage can be induced by certain concentrations of Camptothecin, Cisplatin, and Etoposide, or treated with 100μM hydrogen peroxide or 25μM KMnO4 at 4℃ for 20 minutes.
5. The Propidium Iodide Solution in this reagent kit is harmful to the human body. Please be careful during operation and pay attention to effective protection to avoid direct contact with the human body or inhalation.
6. This product is only for scientific research purposes by professionals and cannot be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residential areas.
7. For your safety and health, please wear laboratory clothes and disposable gloves when operating.

Protocol

1. User provided instruments, consumables, and reagents.

1.1. Instruments and consumables: horizontal electrophoresis instrument, fluorescence microscope, microwave, constant temperature water bath, slide and cover glass.

1.2. Reagent: PBS, neutral buffer (0.4M Tris HCl, pH 7.5, can be prepared using 1M Tris HCl, pH 7.5 (Sterile, DNase free)), electrophoresis buffer (1mM EDTA (pH 8.0), 200mM NaOH, no pH adjustment required, final pH is about 13).

The preparation of electrophoresis buffer is shown in the table below,

Note: Neutral buffer and electrophoresis buffer should be freshly prepared and pre cooled at 4℃.

Reagent	Volume
NaOH	4g
0.5M EDTA	1ml
Ultrapure Water	499ml
Total Volume	500ml

2. Preparation of the reagent kit.

2.1. Preparation of 1% normal melting point agarose gel: calculate the weight of agarose needed according to the concentration and volume of agarose gel, weigh an appropriate amount of Normal Melting Point Agarose and add it into a suitable container (such as a 50ml centrifuge tube), add an appropriate amount of PBS and heat it for dissolution.

For example, add 10ml PBS to 100mg of normal melting point agarose, shake and mix the agarose powder appropriately, heat and boil the agarose solution in a microwave, or place it in a 90-100℃ water bath until completely dissolved. Then place it in a 45℃ water bath for cooling and backup use. The unused 1% normal melting point agarose gel can be stored at 4℃ and used after the next remelting.

Note: If necessary, a mark can be made on the liquid surface to facilitate the use of ultrapure water or deionized water to replenish to the initial volume after some moisture has evaporated.

2.2. Preparation of 0.7% low melting point agarose gel: calculate the weight of the required low melting point agarose according to the concentration and volume of the agarose gel, weigh an appropriate amount of Low Melting Point Agarose and add it into a suitable container (such as a 50ml centrifuge tube), add an appropriate amount of PBS and heat it for dissolution.

For example, add 10ml PBS to 70mg of low melting point agarose, shake well and mix the low melting point agarose powder. Take a water bath at 70-80℃ for 5-10 minutes to completely dissolve the agarose solution. Then cool in a 37℃ water bath for at least 20 minutes before use. The unused 0.7% low melting point agarose gel can be stored at 4℃ and used after the next remelting.

Note: 1)It is important to cool low melting point agarose in a 37℃ water bath for at least 20 minutes, otherwise excessively high temperatures can cause DNA damage.

2)Low melting point agarose is best dissolved in a water bath at 70-80℃, do not use a microwave.

3)Low melting point agarose solution can be maintained in solution state at 37℃ for 1-2 hours.

3. Preparation of samples.

3.1. **Adherent cells:**Collect cell supernatant (including suspended cells), digest adherent cells with trypsin, collect cell suspension, mix with collected cell supernatant, and centrifuge to remove supernatant. Wash the cells with pre cooled PBS in an ice bath once, collect the cell precipitate by centrifugation, add an appropriate amount of PBS and resuspend to achieve a cell density of 1×10^6 cells/ml.

3.2. **Suspended cells:** After collecting the cell suspension, centrifuge to remove the supernatant. Wash the cells once with pre cooled PBS in an ice bath, collect the cell precipitate by centrifugation, and add an appropriate amount of PBS to resuspend to achieve a cell density of 1×10^6 cells/ml.

3.3. **Organizational samples:** Prepare cell suspensions using methods such as trypsin and collagenase digestion, and then refer to suspended cells for operation.

Note: 1)It is best for cells to be freshly prepared. If using frozen cells, it is necessary to first determine whether freezing will cause additional DNA damage to the cells.

2)For each experiment, untreated normal cells need to be set as blank controls (Control).

4. Production.

4.1. **Preparation of the first layer of gel:** this layer is 1% normal melting point agarose gel. Place the frosted side of the slide upward, preheat it at 45℃, and place the preheated 30μl 1% normal melting point agarose gel in the round hole of the comet electrophoresis slide or on the slide, cover the cover slide, and place it at 4℃ for 10 minutes to solidify. Gently remove the cover glass and place it at room temperature for standby. At this time, the gel is a thin layer.

Note:1)When covering with a cover glass, avoid producing bubbles.

2) When removing the cover glass, gently push it to properly shift it, then use pointed tweezers to slowly lift it to avoid breaking the adhesive. After removing the cover slide, place it at room temperature to dry overnight, and the first layer of gel has better wall adhesion effect. An 18×18mm cover glass is easier to remove than a 24×24mm one, and it is recommended to use an 18×18mm cover glass.

3)For the untested slide, it is recommended to ensure that the normal melting point agarose gel does not fall off on the slide. Appropriate ground glass or frosted glass slide can also be used, so that gel will not fall off easily and will not affect subsequent fluorescence observation.

4.2. **Preparation of the second layer of gel:** this layer is low melting point agarose containing cell suspension. Mix 10μl cells (about 10^4 cells) and 75μl 0.7% low melting point agarose in a 37℃ water bath evenly, then quickly absorb 70μl drops and add them to the first layer of gel, gently spread them evenly with a pipette tip, cover the entire first layer of glue, or cover a cover slide, and let it solidify at 4℃ for 10 minutes.

Note: 1) When laying the adhesive, it is necessary to avoid the generation of bubbles. If there are many samples, low melting point agarose can be packed in a 1.5ml centrifuge tube placed in a 37℃ water bath, and then cells can be added and mixed evenly. After mixing, glue should be applied in a timely manner to prevent the glue from solidifying.

2)In general, simply use the pipette suction head to gently spread and spread evenly, without covering with a cover glass. Cover the glass slide, and the second layer of gel may fall off when it is removed later.

4.3. Preparation of the third layer of gel (optional): after solidification of the second layer of gel, drip 0.7% low melting point agarose preheated at 75μl 37℃. Cover with a new cover glass and let it solidify at 4℃ for 30 minutes. Avoid the generation of bubbles when laying glue.

Note: The double-layer gel method can not only make the gel have better adhesion, but also make the cells as many as possible in the same plane, so the dyeing effect is better; The three-layer adhesive has to some extent solved the problem of detachment and floating, but it may affect the observation effect.

5. Cell lysis.

5.1. Preparation of cracking solution: Prepare cracking solution according to the ratio of Lysis Buffer to DMSO at 9:1.

For example, take 9ml Lysis Buffer and add 1ml DMSO, mix well to obtain 10ml lysis solution. After preparation, place at 4℃ for pre cooling and standby.

Note: 1)The cracking solution must be used and prepared on site. The cracking solution may become turbid at room temperature, and after thorough mixing or mixing, it will become clear at 4℃.

2)If red blood cells or heme are present in cell or tissue samples, DMSO can avoid DNA damage caused by reactive oxygen species catalyzed by iron in heme. If the sample does not contain hemoglobin or heme, DMSO can also be omitted from the lysis solution.

5.2. If there is a cover glass, gently remove the cover glass according to step 4a, place the glass slide in a 10cm culture dish, pour about 10ml of precooled pyrolysis solution, and submerge the gel and glass slide. Crack at 4℃ for 1-2 hours or overnight, remove the slide and rinse with PBS for 3 minutes.

6. DNA deconvolution.

Place the slide in a horizontal electrophoresis tank, pour in the electrophoresis buffer, at least 0.25cm above the gel surface of the slide, and let it sit at room temperature for 20-60 minutes. This step causes the double stranded DNA to unwind under alkaline conditions and facilitates the migration of broken small fragments of DNA in an electric field.

7. Electrophoresis.

Electrophoresis can be performed in any horizontal electrophoresis tank, with a low voltage of 25V (~0.75-1V/cm) and a short electrophoresis time of 20-30 minutes.

Note:1) When electrophoresis, it is advisable to use a low temperature, preferably an ice bath, and avoid light. It is recommended to avoid light electrophoresis in a cold storage or 4℃ refrigerator. The electrophoresis time of different electrophoresis tanks needs to be adjusted appropriately to maintain an electric field strength of about 0.75-1V/cm.

2)If the voltage is too high or the electrophoresis time is too long, the DNA of non damaged cells may also show false positive results in comet morphology; On the contrary, if the voltage is too low or the time is too short, the electrophoresis of small fragments of DNA is insufficient, and damaged cells have no comet tails, resulting in false negative results.

8. Neutralization and staining.

After electrophoresis, place the slide in a petri dish, add neutral buffer, immerse the slide, neutralize 1-3 times at 4℃ for 5-10 minutes each time, discard the neutral buffer, and add about 20μl of Propidium Iodide Solution onto the slide. Stain in dark for 10-20 minutes. Then wash with Ultra Water three times, cover with a cover glass, and then observe under a fluorescence microscope.

Note: 1)The combination of gel and slide after cell lysis, DNA unwinding, electrophoresis and neutralization may not be as close as before, so it should be handled with care after electrophoresis and neutralization, and it should be taken out gently horizontally to avoid gel falling off.

2)If a transparent slide is used, it can be neutralized once for 5 minutes. The more times of neutralization, the easier the gel will fall off. Immerse the glass slide in neutral buffer solution, too much will cause gel to fall off.

3)Fluorescent dyes such as EB, DAPI, SYBR Gold, SYBR Green, NA Red, Gel Red, NA Green, Gel Green, or silver staining can also be used for staining, and corresponding detection methods can be used for detection.

9. Observation, photography, and analysis of experimental results.

9.1. Observation and photography: Under a fluorescence microscope, the Propidium Iodide exhibits red fluorescence with Ex/Em=535/617nm.

9.2. **Result analysis:** The common characteristic indicators of DNA damage measured by alkaline comet electrophoresis include comet cell rate, distance indicators include comet total length, tail length, comet head radius, comet tail radius, etc., intensity indicators include head DNA percentage, tail DNA percentage, head area, tail area, etc., and moment indicators include tail moment, etc. Length of Tail, which refers to the difference in migration length between undamaged large molecule DNA and damaged small molecule DNA (Tail in Figure 2), shows a linear relationship with DNA damage within the low damage dose range; Percentage of DNA in the Tail (Tail DNA%) is the percentage of tail DNA in the total cell DNA; Tail Moment, which is the product of tail length and the percentage content of tail DNA, shows a linear relationship with the degree of damage at high injury doses. It combines the amount of tail DNA with the migration distance relative to the nucleus. The two most important indicators are the percentage content of tail DNA and tail moment, which are calculated as follows:

Tail DNA (%) = 100×Tail DNA Intensity/Cell DNA Intensity Tail Moment

There are two calculation methods:

1. Olive Tail Moment=Tail DNA (%)×Tail Moment Length*
2. Extent Tail Moment=Tail DNA (%)×Length of Tail

***Tail Moment Length is measured from the center of the head to the center of the tail (Refer to Figure 2).**

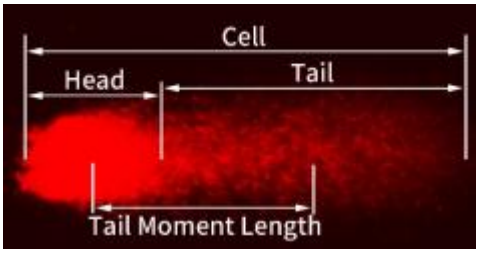

Figure 2. Tail moment of cellular DNA damage in comet electrophoresis.

9.3. DNA damage assessment: Randomly select 50-100cells from each sample and measure the corresponding indicators. DNA damage is divided into 5 levels based on the percentage of tail DNA, which can be used:

Level 0:<5% without damage;

Level 1: 5-20% mild injury;

Level 2: 20-40% moderate injury;

Level 3: 40-95% high damage;

Level 4:>95% severe injury.