Comet Assay Kit

Comet Assay Kit is an assay kit for the quantification of Comet in cells.

Catalog number: ARG83611

Package: 96 assay

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INTRODUCTION

DNA damage, due to environmental factors and normal metabolic processes inside the cell, occurs at a rate of 1,000 to 1,000,000 molecular lesions per cell per day. While this counts for only a small part of the human genome's approximately 6 billion bases (3 billion base pairs), unrepaired lesions to critical genes can impede a cell's ability to carry out its function and appreciably increase the likelihood of cancer.

The comet assay, or single cell gel electrophoresis assay (SCGE), is a common technique for measurement of DNA damage in individual cells. Under an electrophoretic field, damaged cellular DNA (containing fragments and strand breaks) is separated from intact DNA, yielding a classic “comet tail” shape under the microscope. Extent of DNA damage is usually visually estimated by comet tail measurement; however, image analysis software is also available for measuring various parameters.

PRINCIPLE OF THE ASSAY

Comet Assay Kit is a single cell gel electrophoresis assay (SCGE) for simple evaluation of cellular DNA damage. First, individual cells are mixed with molten agarose before application to the 96-Well Comet Slide. These embedded cells are then treated with a lysis buffer and alkaline solution, which relaxes and denatures the DNA. Finally, the samples are electrophoresed in a horizontal chamber to separate intact DNA from damaged fragments. Following electrophoresis, the samples are dried, stained with a DNA dye, and visualized by epifluorescence microscopy. Under these conditions, the damaged DNA

(containing cleavage and strand breaks) will migrate further than intact DNA and produce a “comet tail” shape.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store the Vista Green DNA Dye at -20°C. Store all other components at room temperature. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 x 96-well plate	RT
Comet Agarose	15 mL	RT
10000X Vista Green DNA Dye	5 µL	-20°C(Protect from light)
EDTA Solution,	1 vial (50 mL; 500 mM)	RT
10X Lysis Solution	20 mL	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- NaCl powder
- NaOH pellets
- 10 N NaOH for pH adjustment
- DMSO (optional)
- 70% Ethanol
- TE Buffer (10 mM Tris, pH 7.5, 1 mM EDTA)
- PBS (without Mg^{2+} and Ca^{2+}) and DI H_2O
- EDTA (disodium salt)
- Pipettes and pipette tips

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection.
- Upon received, store the Vista Green DNA Dye at -20°C. Store all other components at room temperature. Use the kit before expiration date.
- Briefly spin down the reagents before use.
- Change pipette tips between the addition of different reagent or samples.
- To avoid ultraviolet light damage to cell samples, perform the assay under low/dim light conditions.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Suspension Cells- Centrifuge cells at 700 x g for 2 minutes and discard supernatant. Wash cell pellet once with ice-cold PBS (without Mg^{2+} and Ca^{2+}), centrifuge, and discard the supernatant. Finally, resuspend the cells at 1 x 105 cells/mL in ice-cold PBS (without Mg^{2+} and Ca^{2+}).

Adherent Cells- Gently remove cells from flask/dish by scraping with a rubber policeman. Transfer cell suspension to a conical tube and centrifuge at 700 x g for 2 minutes, discarding the supernatant. Wash cell pellet once with ice-cold PBS (without Mg^{2+} and Ca^{2+}), centrifuge, and discard the supernatant. Finally, resuspend the cells at 1 x 105 cells/mL in ice-cold PBS (without Mg^{2+} and Ca^{2+}).

Tissue Preparation- Using dissection scissors, mince a small piece of tissue in 1-2 mL of ice-cold PBS containing 20 mM EDTA (without Mg^{2+} and Ca^{2+}). Allow the tissue/cell suspension to stand for 5 minutes before transferring the supernatant to a centrifuge tube; avoid transferring debris. Centrifuge, discarding the supernatant, and then resuspend the cells at 1 x 105 cells/mL in ice-cold PBS (without Mg^{2+} and Ca^{2+}).Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- Comet Agarose: Heat the Comet Agarose bottle at 90-95°C in a water bath for 20 minutes, or until agarose liquefies. Transfer the bottle to a 37°C water bath for 20 minutes and maintain until needed.
- 1X Vista Green DNA Staining Solution: Dilute the 10000X Vista Green DNA Dye with TE Buffer (10 mM Tris, pH 7.5, 1 mM EDTA) to yield 1X Vista Green DNA Staining Solution.
- 1X Lysis Buffer (100 mL)

Component	Quantity
NaCl	14.6 g
EDTA Solution (provided)	20.0 mL
10X Lysis Solution (provided)	10.0 mL
DMSO	10.0 mL (optional for heme containing samples)
DI H_2O	Adjust volume to 90 mL

Mix thoroughly to dissolve NaCl. Slowly adjust the Lysis Buffer to pH 10.0 with 10 N NaOH, then QS to 100 mL with DI H_2O. Chill Lysis Buffer to 4ºC

before use.

Note: Buffer will appear cloudy at room temperature, but will clear at 4°C. pH will also remain ~10.0

- Alkaline Solution (100 mL)

Component	Quantity
NaCl	1.2 g
EDTA Solution (provided)	0.2 mL
DI H_2O	Adjust volume to 100 mL

Mix thoroughly to dissolve NaOH. Chill Alkaline Solution to 4°C before use.

- Electrophoresis Running Solution: Choose the appropriate electrophoresis solution based on the desired running conditions and assay sensitivity.

TBE Electrophoresis Solution(1 L)

Component	Quantity
Tris Base	10.8 g
Boric Acid	5.5 g
EDTA (disodium salt)	0.93 g
DI H_2O	Adjust volume to 1 L

Mix thoroughly to dissolve solids. Chill TBE Running Solution to 4°C before use.

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

Component	Quantity
NaOH	12.0 g
EDTA Solution (provided)	2.0 mL
DI H_2O	Adjust volume to 1 L

Mix thoroughly to dissolve NaOH. Chill Alkaline Running Solution to 4°C before use.

- Combine cell samples with Comet Agarose (see Preparation of Reagents) at 1:10 ratio (v/v). Mix well by pipetting, and immediately transfer 20

µL/well onto the Comet Slide using a multichannel micropipette. Ensure complete well coverage by spreading the solution over the well with the pipette tip.

Note: For multiple samples, warm the slide at 37°C, maintain suspensions at 37°C to avoid gelation. Titrate samples again just prior to slide addition.

- Maintaining the slide horizontally, transfer the slide to 4°C in the dark for 15-30 minutes.
- Carefully, transfer the slide to a small basin/container containing pre-chilled Lysis Buffer (~50-100 mL/slide). Immerse the slide in the buffer for 30-60 minutes at 4°C in the dark.
- Carefully, aspirate the Lysis Buffer from the container and replace with pre-chilled Alkaline Solution (~50-100 mL/slide). Immerse the slide in the solution for 30 minutes at 4°C in the dark.

ASSAY PROCEDURE

- TBE Electrophoresis is preferred for analysis of apoptosis and enables use of the tail length, rather than the tail moment, for data analysis. TBE electrophoresis will detect single-stranded and double-stranded DNA breaks, and may detect a few AP sites.
    1. Aspirate the Alkaline Solution from the container and replace with pre-chilled TBE Electrophoresis Solution. Immerse the slide for 5 minutes, and then repeat once more.
    2. Maintaining the slide horizontally, carefully transfer the slide to a horizontal electrophoresis chamber. Fill the chamber with cold TBE Electrophoresis Solution until the buffer level covers the slide.

3. Apply voltage to the chamber for **10-15 minutes** at **1 volt/cm** (e.g., if the chamber electrodes are **35 cm** apart, you would then apply **35 volts** to the slide)
4. Maintaining the slide horizontally, carefully transfer the slide from the electrophoresis chamber to a clean, small basin/container containing pre-chilled **DI H_2O** (~50-100 mL/slide). Immerse the slide for **2 minutes**, aspirate, and then repeat twice more.
5. Aspirate the final water rinse and replace with **cold 70% Ethanol** for **5 minutes.**
6. Maintaining the slide horizontally, remove the slide from the **70% Ethanol** and allow slide to dry at **37°C** for **30 min**.
7. Once the agarose and slide are completely dry, add **50 µL/well** of **diluted Vista Green DNA Dye** (see Preparation of Reagents). Incubate at room temperature for **15 minutes**.
8. View slides by epifluorescence microscopy using a FITC filter.

- **Alkaline electrophoresis** is more sensitive and will detect smaller amounts of DNA damage. Alkaline electrophoresis will detect single-stranded and double-stranded DNA breaks, the majority of AP sites, and alkali labile DNA adducts
    1. Maintaining the slide horizontally, carefully transfer the slide from the Alkaline Solution to a horizontal electrophoresis chamber. Fill the chamber with **cold Alkaline Electrophoresis Solution** until the buffer level covers the slide.
    2. Apply voltage to the chamber for **15-30 minutes** at **1 volt/cm** (e.g., if the chamber electrodes are **35 cm** apart, you would then apply **35**

volts to the slide). Additionally, adjust the volume of Alkaline Electrophoresis Solution to produce a current setting of 300 mA.

3. Maintaining the slide horizontally, carefully transfer the slide from the electrophoresis chamber to a clean, small basin/container containing pre-chilled DI H_2O (~50-100 mL/slide). Immerse the slide for 2 minutes, aspirate, and then repeat twice more.
4. Aspirate the final water rinse and replace with cold 70% Ethanol for 5 minutes.
5. Maintaining the slide horizontally, remove the slide from the 70% Ethanol and allow slide to dry at 37°C for 30 min.
6. Once the agarose and slide are completely dry, add 50 µL/well of diluted Vista Green DNA Dye (see Preparation of Reagents). Incubate at room temperature for 15 minutes.
7. View slides by epifluorescence microscopy using a FITC filter.

CALCULATION OF RESULTS

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% = 100 x Tail DNA Intensity/Cell DNA Intensity

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

(a) Olive Tail Moment = Tail DNA% x Tail Moment Length*

(b) Extent Tail Moment = Tail DNA% x Length of Tail

A number of Comet Assay analysis software programs are commercially available, such as and Comet Assay IV (Perceptive Instruments) and CASPlab.

*Tail Moment Length is measured from the center of the head to the center of the tail