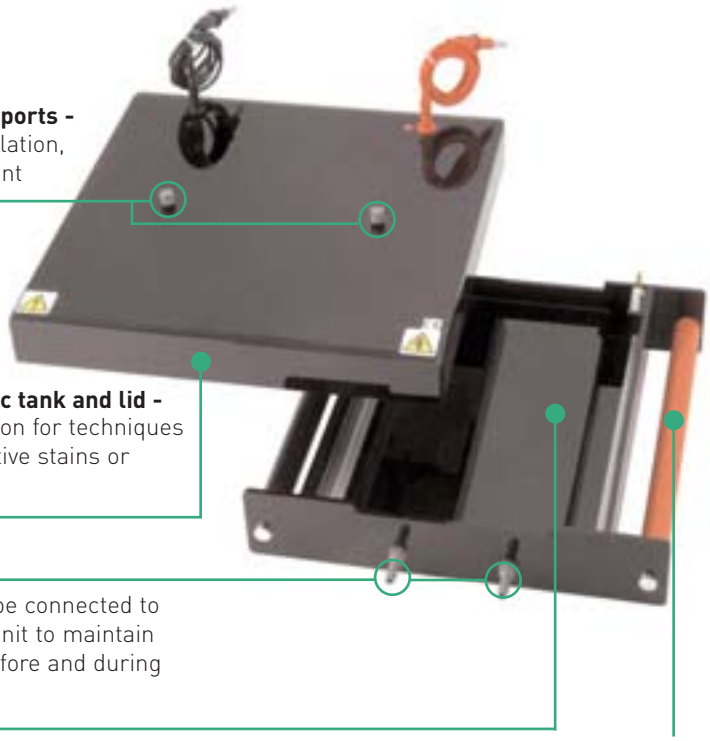
COMET Single Cell Gel Electrophoresis Systems

The COMET single cell gel electrophoresis systems are designed specifically for single cell gel electrophoresis (SCGE). Comet Assays are used to detect and quantify DNA damage and repair within individual cells in genetic toxicology and carcinogenesis studies.

BENEFITS INCLUDE

- **Available in 4 formats -** to accommodate 10, 20, 40 and 80 standard microscope slides respectively
- **Opaque ebony acrylic tank and lid -** provide ideal protection for techniques requiring light-sensitive stains or samples
- **Cooled central platform -** provides a convenient surface for slide preparation in addition to maintaining slide temperature during pre-incubation and electrophoresis (see below)
- **Cooling ports -** can be connected to the JULABO chiller unit: to prevent overheating during SCGE/Comet assays, typically performed at high current settings over 300mA; and to inhibit DNA repair enzyme activity by maintaining the slide temperature at 4°C, either during preparation and mounting or pre-incubation and electrophoresis
- **Buffer recirculation ports -** can be connected to a peristaltic pump to maintain buffer circulation, preventing ion gradient formation
- **Colour-coded handles -** corresponding to the anode and cathode - serve as a visual aid to ensure that the slides are positioned in the correct polar orientation for the assay



Buffer recirculation ports - maintain buffer circulation, preventing ion gradient formation

Opaque ebony acrylic tank and lid - provide ideal protection for techniques requiring light-sensitive stains or samples

Cooling ports - can be connected to the JULABO chiller unit to maintain slide temperature before and during electrophoresis

Cooled central platform - provides a convenient surface for slide preparation in addition to maintaining slide temperature, through a labyrinthine cooling base, during pre-incubation and electrophoresis

Colour-coded handles - corresponding to the anode and cathode - serve as a visual aid to ensure that the slides are positioned in the correct polar orientation for the assay

TECHNICAL SPECIFICATION

	COMET-10	COMET-20	COMET-40	COMET-80
Unit Dimensions (W x L x H)	31 x 23.5 x 6.5cm	31 x 32 x 6.5cm	31 x 47.5 x 6.5cm	31 x 77.5 x 6.5cm
Active Tank Dimensions (W x L x H)	27.5 x 13 x 3.5cm	27.5 x 21.5 x 3.5cm	27.5 x 37 x 3.5cm	27.5 x 67 x 3.5cm
Recommended Buffer Volume (ml)	450ml	600ml	800ml	1200ml
Buffer Recirculation Ports	2	2	2	2
Slide Capacity (25 x 75mm; W x L)	10	20	40	80
Recommended Running Conditions	5V/cm (300mA)	5V/cm (300mA)	5V/cm (300mA)	5V/cm (300mA)
Power Output Connectors (diameter)	Shrouded, 4mm	Shrouded, 4mm	Shrouded, 4mm	Shrouded, 4mm
Recommended Power Supplies	Consort EV261	Consort EV261	Consort EV261	Consort EV261

THE COMET ASSAY

Background - The Comet Assay/SCGE was pioneered by Östling and Johansson (1) in 1984 as a neutral pH assay to quantify double-stranded DNA breakages (DSBs) in single cells exposed to γ irradiation. Singh et al. (2) then modified the assay in 1988 to a more versatile and sensitive alkaline method to measure both single- (SSBs) and double-stranded DNA breakages. Now, by introducing subtle changes to the assay conditions (pH/temperature), the Comet Assay can be tailored to analyse specific DNA lesions and repair processes.

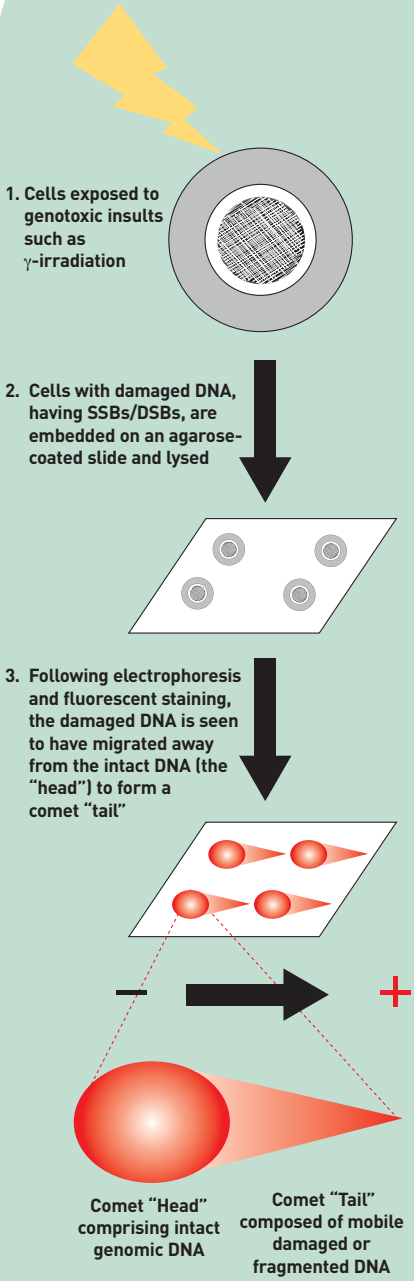
Overview - The Comet Assay is based on the principle that strand breakage of supercoiled duplex DNA reduces the size of the large genomic DNA molecule from which these strands are separated or stretched out by electrophoresis. The high pH of the alkaline lysis step causes denaturation, unwinding of the duplex DNA and the release of alkali labile sites as SSBs. These then become "comets" as the broken ends of the negatively charged DNA molecule migrate towards the anode during electrophoresis.

Method - Cells, previously exposed to a genotoxic insult, are suspended in low melting point agarose (LMP) and embedded within a thin agarose gel on a microscope slide. All cellular proteins are then removed by lysis in detergent solution, when the DNA is allowed to unwind in the neutal/alkaline pH conditions of the detergent. The DNA is electrophoresed and then stained with a DNA-specific fluorescent dye such as acridine orange, ethidium bromide, propidium iodide or 4',6'-diamidino-2-phenylindole (DAPI).

Result - Upon staining cellular DNA is measured for fluorescence, usually with a microscopy imaging system. The resulting image resembles a "comet", with the cellular DNA segregating into a "head" and "tail". The head is composed of largely intact genomic DNA, while the tail comprises damaged (SSBs, DSBs) or fragmented DNA, with the fluorescence intensity and length of the tail being directly proportional to the extent of the DNA damage.

References - 1. Östling, O., and Johanson, K.J., Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochem. Biophys. Res. Commun., 123, 291-8 (1984).

2. Singh, N.P, et al., A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res., 175, 184-91 (1988).



ORDERING INFORMATION

Comet Single Cell Gel Electrophoresis Systems Complete System	Part No.
Comet assay system for ten 75 x 25mm slides, including 27.5 x 13cm tank and lid made in ebony acrylic	COMET-10
Comet assay system for twenty 75 x 25mm slides, including 27.5 x 21.5cm tank and lid made in ebony acrylic	COMET-20
Comet assay system for forty 75 x 25mm slides, including 27.5 x 37cm tank and lid made in ebony acrylic	COMET-40
Comet assay system for eighty 75 x 25mm slides, including 27.5 x 67cm tank and lid made in ebony acrylic	COMET-80

Replacement Parts & Accessories	
2 x 1 metre power leads with shrouded 4mm power output connectors	CABLE-4

Chemicals & Reagents	
1 x 100g Agarose SERVA low melting research grade	11408.03
1 x 100g Ethylenediamine tetraacetic acid.Na_2-salt	11280.01
1 x 1kg Boric acid analytical grade	15165.01
1 x 1kg Tris(hydroxymethyl)aminomethane	37190.02
1 x 250ml Triton® X-100 molecular biology grade	39795.02
1 x 1kg Sodium dodecyl sulphate pellets	20765.03
1 x 50g Acridine orange research grade	10665.02
1 x 5g Ethidium bromide research grade	21238.02
1 x 25mg Propidium iodide research grade	33671.01
1 x 10mg 4',6'-diamidino-2-phenylindole.2HCl.H_20 (DAPI) analytical grade	18860.01

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