Instruction Manual

Comet Assay Tanks

CSL-COM10 CSL-COM20
CSL-COM40 CSL-COM80

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SAFETY PRECAUTION



WHEN USED CORRECTLY, THESE UNITS POSE NO HEALTH RISK.
HOWEVER, THESE UNITS CAN DELIVER DANGEROUS LEVELS OF ELECTRICITY AND ARE TO BE OPERATED ONLY BY QUALIFIED PERSONNEL FOLLOWING THE GUIDELINES LAID OUT IN THIS INSTRUCTION MANUAL.

ANYONE INTENDING TO USE THIS EQUIPMENT SHOULD READ THE COMPLETE MANUAL THOROUGHLY.

THE UNIT MUST NEVER BE USED WITHOUT THE SAFETY LID CORRECTLY IN POSITION.
THE UNIT SHOULD NOT BE USED IF THERE IS ANY SIGN OF DAMAGE TO THE EXTERNAL TANK OR LID.

THESE UNITS COMPLY WITH THE STATUTORY CE SAFETY DIRECTIVES:
73/23/EEC: LOW VOLTAGE DIRECTIVE: IEC 1010-1:1990 plus AMENDMENT 1:1992
EN 61010-1:1993/BS EN 61010-1:1993

PACKING LISTS:

	Main Unit and Lid	Connecters	Buffer Recirculation Ports	Instruction Manual
CSL-COM10		CSL-CAB	CSL-BRP	
CSL-COM20		CSL-CAB	CSL-BRP	
CSL-COM40		CSL-CAB	CSL-BRP	
CSL-COM80		CSL-CAB	CSL-BRP	

The packing lists should be referred to as soon as the units are received to ensure that all components have been included. The unit should be checked for damage when received.

Please contact your supplier if there are any problems or missing items.

Usage Guidance and restrictions:

- Maximum altitude 2,000m.
- Temperature range between 4°C and 65°C.
- Maximum relative humidity 80% for temperatures up to 31^{O}C decreasing linearly to 50% relative humidity at 40^{O}C.
- Not for outdoor Use.

This apparatus is rated POLLUTION DEGREE 2 in accordance with IEC 664. POLLUTION DEGREE 2, states that: “Normally only non-conductive pollution occurs.
Occasionally, however, a temporary conductivity caused by condensation must be expected”.

Care and Maintenance:-

Cleaning Comet Assay Units

Units are best cleaned using warm water and a mild detergent. **Water at temperatures above 60^0 C can cause damage to the unit and components.**

The tank should be thoroughly rinsed with warm water or distilled water to prevent build up of salts but care should be taken not to damage the enclosed electrode and vigorous cleaning is not necessary or advised.

Air drying is preferably before use.

The units should only be cleaned with the following:-

Warm water with a mild concentration of soap or other mild detergent.

Compatible detergents include dishwashing liquid, Hexane and Aliphatic hydrocarbons

The units should not be left to in detergents for more than 30 minutes.

The units should never come into contact with the following cleaning agents, these will cause irreversible and accumulative damage:-

Acetone, Phenol, Chloroform, Carbon tetrachloride, Methanol, Ethanol, Isopropyl alcohol

Alkalis.

RNase Decontamination

This can be performed using the following protocol:-

Clean the units with a mild detergent as described above.

Wash with 3% hydrogen peroxide (H2O2) for 10 minutes.

Rinsed with 0.1% DEPC- (diethyl pyrocarbonate) treated distilled water,

Caution: DEPC is a suspected carcinogen. Always take the necessary precautions when using. RNaseZAP™ (Ambion) can also be used. Please consult the instructions for use with acrylic gel tanks.

Setting up the Comet Assay Tanks:-

Instructions for fitting Electrode Cables.

1. Note the position of the lid on the unit. This shows the correct polarity and the correct orientation of the cables, black is negative and red positive.
2. Remove the lid from the unit. Note if the lid is not removed, fitting the cables may result in un-tightening of the gold plug and damage to the electrode.
3. Screw the cables into the tapped holes as fully as possible so that there is no gap between the lid and the leading edge of the cable fitting.
4. Refit the lid.

Connecting to the Chiller Unit

1. The Comet Assay is a high current technique. High current may cause the build-up of heat which can affect assay resolution. To counteract the adverse effects of heat generation, Cleaver Scientific recommends using an effective chiller unit, such as the CSL-CHILLER, preset to 4°C.
2. For active temperature regulation attach two short lengths of hose from the inlet and outlet ports of the chiller unit to the respective outlet and inlet connectors within the cooling platform.
3. Ensure that the flow rate of the chiller unit is set at a maximum 1L/minute. Do not exceed this limit as damage to the cooling platform might occur.
4. If using a chiller unit which exceeds this limit, a T-connector may be used. The T-connector can divert some water volume back to the recirculating chiller reducing the water pressure incident within the cooling platform.

The unit is now ready to be used.

Protocol:

Preparation of Cells

1. Collect the cells by scraping into the media solution. Do not use trypsin as this causes damage to DNA.
2. Pellet the cells and resuspend them in PBS. The recommended amount of cells per slide is 10,000 cells and these should be added to each slide in a 10µl volume. The cells may be counted using a haemocytometer.

Preparation of Slides

3. Pipette 110µl of Standard Melting Point agarose (0.5% in PBS) to a polylysine-coated microscope slide.
4. Carefully place over the agarose a coverslip and chill the slide at 4°C for around 10-15 minutes to allow the agarose to solidify.
5. Carefully remove the coverslip.
6. Mix together 65µl of Low Melting Point agarose (at 37°C) with 10µl of the cell suspension.
7. Place the agarose/cell mixture on top of the previous layer of agarose on the slide.
8. Cover with a coverslip and return to chill at 4°C until solid.
9. Carefully remove the coverslip and add 75µl of Standard Melting Point agarose onto the slide.
10. Cover with a coverslip and return to chill at 4°C until solid.

Lysis and Electrophoresis

All steps should be done with **minimal exposure to light** to reduce background DNA damage (i.e. in a darkroom under yellow light, or wrapped in foil on the bench)

11. Remove the coverslips and place slides in chilled lysis solution at 4°C for one hour.
12. Remove the slides from lysis solution and place in the Comet Assay tank.
13. Fill the tank with just enough electrophoresis buffer to completely cover the slides, keeping them in position on the cooling platform. Cleaver Scientific recommends the following buffer volumes: -

Unit	**CSL-COM10**	**CSL-COM20**	**CSL-COM40**	**CSL-COM80**
Volume (ml)	550ml	1000ml	2100ml	4000ml

14. Leave the slides in electrophoresis buffer for 20-30 minutes to allow the DNA to unwind.
15. Electrophorese at 25V for around 20 minutes. Resultant current should be no more than 300 mA.

Analysis of slides

16. Neutralise the slides by slowly washing with Neutralisation Buffer.
17. Add 25 µl of Ethidium Bromide (20 µg/ml) to each slide and cover with a coverslip.
18. Examine the slides under a fluorescent microscope.

Solutions:-

Lysis solution

2.5M NaCl, 100 mM EDTA, 10 mM Tris, pH10

1% Triton X-100 added fresh

Electrophoresis Buffer

1 mM EDTA, 300 mM NaOH

Neutralisation Buffer

0.4 M Tris, pH 7.5

Notes

Warranty

The Cleaver Scientific Ltd. (CSL) Comet Assay units have a warranty against manufacturing and material faults of twelve months from date of customer receipt. If any defects occur during this warranty period, CSL will repair or replace the defective parts free of charge.

This warranty does not cover defects occurring by accident or misuse or defects caused by improper operation.

Units where repair or modification has been performed by anyone other than CSL or an appointed distributor or representative are no longer under warranty from the time the unit was modified.

Units which have accessories or repaired parts not supplied by CSL or it's associated distributors have invalidated warranty.

CSL cannot repair or replace free of charge units where improper solutions or chemicals have been used. For a list of these please see the Care and Maintenance subsection.

If a problem does occur then please contact your supplier or CSL on:-

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