



LOCATION DETAILS

School/Branch: School of Molecular & Biomedical Science

SAFE OPERATING PROCEDURE DETAILS

Constant Systems Cell Disruptor

Date Prepared:
26/10/2010

PREPARED BY: Name, position, & Signature (insert names of supervisor, HSO, subject matter expert)

Tony Richardson – Infrastructure Manager
Chris McDevitt – Research Fellow
Kate Millan – Health & Safety Officer

Signature:

RISK ASSESSMENT

Has a risk assessment been completed and all other environmental considerations been made?
YES

See risk assessment dated:
20/09/2010

Risk Rating:
~~Low~~
Medium
High
Very High

RISKS IDENTIFIED

- High Pressure (Hydraulic)
- Moving (possible ejection) metal parts
- Hot Metal (up to 50° C)
- Aerosols of Microorganisms at the disruption head outlet

SAFETY PRECAUTIONS

The following control measures **MUST** be adhered to:

- **Pressure limits stated in the manual must be observed**
- **The power must be disconnected (not just switched off) when any part of the machine is dismantled**
- **Avoid touching the disruption head when in operation**
- **All pathogenic and genetically modified material must be contained to PC2 standards**
- **Disinfection of the disruption head and working surfaces after use**

PERSONAL PROTECTIVE EQUIPMENT REQUIRED

The following PPE must be worn at all times:

- Lab Gown,
- eye protection
- Also gloves when appropriate

SAFE OPERATING PROCEDURE

Before using the Constant Systems Cell Disruptor users must be trained and logged by one of the following:

- Renato Morona
- Chris McDevitt

Considerations

- **Cell Concentration:** The cell disruptor can break cells at concentrations up to 25g/Litre **BUT: cells must very well homogenised.**

- **Foreign material:** It is vital all non-soluble material (e.g. glass, foil, etc.) is removed. This material will irreversibly block the inlet jet and require expensive replacement.
- **Frothing agents:** must be avoided (**no lysozyme**). Protease inhibitors (**no PMSF**) can cause frothing and so should be added immediately after cell disruption.
- **Aerosols:** If pathogenic and/or genetically modified organisms are used: the outlet must feed into a closed container vented via a 0.2 micron pore hydrophobic filter.
- **Hot Bits:** The hydraulic liquid is heated by the repetitive pressurisation so parts of the disrupter head could become hot - **avoid contact**.

Operation Procedure

1. Switch on chiller and cell disruptor. Allow the chiller to reach 5°C.
2. Select continuous run from the “operations” page
3. Select the pressure using the knob on the front of the machine (do not go above 40K psi)

Recommended pressures for Escherichia coli are:
Below 15,000 psi (1000 Bas) – Release of DNA
15,000 – 20,000 psi (1,350 Bar) – Cytoplasmic Protein
20,000 – 27,000 psi (1900 Bar) – Inclusion Bodies
27,000 – 35,000 psi 2,400 Bar) – Membrane Protein

4. Place sample(minimum volume 25ml) into inlet cup cover with one half of a plastic Petri dish
5. Start run
6. Collect disrupted sample in a suitable container (e.g. 500 “Schott Bottle”). In the case of pathogenic and/or genetically modified samples the container must be an approved aerosol containment system.
7. Wash through with sufficient buffer to remove any retained sample (~ 10 – 50ml)
8. The cell disruptor will automatically stop after sensing 3 empty passages (i.e. no sample). An automatic stop can also occur if the sample is too viscous or frothy.
9. Record usage in the log book with the stroke number and any comments.
10. Clean and sterilise the disruptor:

Cleaning Procedure

- a. Fill the inlet cup 200ml of 0.45 filtered MQ H₂O and passage at 10K psi.
- b. Fill the inlet cup 200ml of 0.45 filtered 70% w/w ethanol and passage at 10K psi.
- c. Fill the inlet cup 200ml of 0.45 filtered MQ H₂O and passage at 10K psi.
 - **Note:** If pathogenic and/or genetically modified organisms are used: the outlet must feed into a closed container vented via a 0.2 micron pore hydrophobic filter.

11. Switch off the cell disruptor and Chiller.

Tony Richardson
27/10/2010

OTHER INFORMATION
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ADMINISTRATION
Note: This Safe Operating Procedure must be reviewed : a) after any accident, incident or near miss; b) when training new staff; c) if adopted by new work group; d) if equipment, substances or processes change; or e) within 5 years of date of issue.