

Standard Operating Procedure

SOP124

Title: Use and Maintenance of the Galaxy 170R CO₂ incubator

Location: CBE (H23)

1. PURPOSE

The purpose of this SOP is to describe the procedures for the use and maintenance of the Galaxy 170R CO₂ incubator, in order to ensure its correct use for the incubation of a variety of vessels containing animal cell lines, human cell lines (including human embryonic cell lines and iPS cell lines) or enzymatic assay material under environmental operating conditions nominally set at 37C and 5% CO₂.

2. SCOPE

This SOP applies Galaxy 170R CO₂ incubator located in the Containment Level 2 CBE Laboratory Unit (H23). This document describes the procedure for the use, maintenance and temperature calibration of the incubator.

Important Restrictions:

This SOP does not cover the set and up and installation of this equipment, details of which can be found in the operating instructions (located in the CBE office). This SOP does not apply to the use and operation of the incubator under hypoxic conditions (0.1-4% O₂). **The use of O₂/N₂ within the incubator chamber requires connection of O₂ and/or N₂ gas supply.**

3. RESPONSIBILITES

3.1. Laboratory Personnel:

- (i) Shall complete proper training before using the incubator.
- (ii) Shall ensure that they are familiar with the incubator, its controls, and emergency procedures by reference to this SOP and the Manufacturer's Operating Instructions.
- (iii) Shall ensure that the incubator is suitable for the work they intend to carry out before commencing.
- (iv) Shall carry out the routine inspection and maintenance of the incubator, as required.
- (v) Shall notify the Laboratory Manager/Responsible Person record any adverse events and alarms that indicate non-conformance or malfunction on the.

3.2. The Responsible Person/Laboratory Manager:

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- (i) Shall ensure laboratory personnel are given suitable information, instruction, training and supervision in the correct use and maintenance of the incubators. The requirements for competence to use the incubators should include instruction in the appropriate and inappropriate use, safe working procedures, calibration and decontamination procedures.
- (ii) Shall coordinate routine inspection and maintenance duties to be performed by laboratory personnel (according to SOP004).
- (iii) Shall investigate any reported problems, adverse event, alarms or non-conformities associated with incubator usage.
- (iv) Shall organise the maintenance, repair or servicing of the incubators by trained and authorised contract / service personnel.
- (v) Shall ensure that prior to authorising the commencement of maintenance, repairs or servicing that the incubator is suitably disinfected and that a "Decontamination Certificate" is issued.

4. EQUIPMENT AND MATERIALS

- 1) Galaxy 170R CO₂ incubator
- 2) Copper sulphate
- 3) 70% (v/v) IMS solution
- 4) Sterile water
- 5) Tinytag temperature data loggers

5. PROCEDURE

5.1 Operation and Use

The incubator should always be switched on, if you find it switched off please contact the responsible person or laboratory manager. The on / off switch is located on the rear of the incubator.

Before adding or removing a culture vessel or equipment (e.g. spinner flask platform) check to ensure the following:

- (i) The screen on the door is on and displaying CO₂ and temperature readings (if this is not the case contact the laboratory manager or responsible person for advice). The temperature should be set to 37°C with an acceptable fluctuation of $\pm 1.0^{\circ}\text{C}$. The CO₂ level should be set to 5%.
 - a. To set the desired operating temperature and CO₂ level press the "**PROG**" function key.
 - b. In the "**PROG**" screen that appears, press the desired function key, "**TEMP**" or "**CO₂**" and then use the direction keys to adjust the value.
 - c. When the desired setpoint is displayed, press the "**ENTER**" function key.

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- d. After making the adjustments (if any were made), allow the incubator to stabilise at the setpoints before continuing.
- (ii) The vessel or equipment is not dirty or contaminated with spilt reagents; in this event clean with 1:50 Chemgene before putting inside the incubator.
- (iii) All culture vessels to be entered into the incubator are labelled (refer to SOP005).

5.2 Preventative Maintenance

NOTE: The incubator is self-maintaining once set up. No setup modifications should be made unless required as part of the calibration schedule.

5.2.1. Weekly inspections

- (i) Regularly check the “**DATALOGGER**” screen for any alarms or events that may have occurred overnight. The “**DATALOGGER**” screen is accessed through the “**USER**” menu. The “**DATALOGGER**” screen provides information about the various alarm events and graphs of parameters recorded.
- (ii) Check the water level at the bottom of the incubator. If required refill the tray with autoclaved, purified water to a volume of at least 1.5 litres, but no more than 2.5 litres. Ensure 0.1% (w/v) copper sulphate is added.
- (iii) Check the gauges on the CO₂ cylinders. If any of the gauges read empty, inform the laboratory Manager or the Responsible Person and follow their instructions.
- (iv) Inspect the incubator for any visible microbial or fungal growth (or smell). If any growth is observed, **follow the procedure described in section 5.2.2** and also ensure that any equipment inside is decontaminated with 70% IMS. Ensure that all culture vessels are thoroughly checked for potential contamination.

5.2.2. Fortnightly cleaning and decontamination procedure

As a minimum, the incubator should be fully cleaned and decontaminated fortnightly. If the experimental set-ups allow it, this should be done weekly.

- (i) Remove all shelving and removable accessories from the incubator.
- (ii) Remove reservoir and dispose of copper sulphate solution, by putting into a suitable container to allow evaporation, and the remainder to be absorbed with absorbent material and disposed of via the yellow stream waste route.
- (iii) Wipe down the interior of the incubator, and all shelving etc, before placing back into the incubator.
- (iv) Refill the reservoir with at least 1.5 litres of copper sulphate solution (1 g per 1L of ultra pure water)

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- (v) Ensure that the temperature and CO₂ levels in the incubator are rising, before leaving the incubator for approximately 1 h.
- (vi) If after 1 h, the incubator has achieved the required temperature and CO₂ set points (nominally 37°C and 5% CO₂) culture vessels can be transferred back into the incubator after being wiped with 1:50 Chemgene.
- (vii) If the incubator has failed to reach either of the set points, the Responsible Person should be notified immediately.
- (viii) Record maintenance procedures on the Maintenance of Equipment log and also the house keeping log.

5.2.2.1 Removal/Reinstallation of O₂ sensor:

- (i) To remove and replace the oxygen sensor, you will need the pentagonal sensor removal tool provided. This is stored in the top draw by the water bath in H23.
- (ii) Pull the rear access cover off the rear outside wall of the incubator to gain access to the oxygen sensor.
- (iii) Reach inside and disconnect the sensor by unplugging the connector. Be sure to grasp the white connector body, not the wire leads.
- (iv) Using the sensor removal tool, unscrew the oxygen sensor by turning it counter-clockwise.
- (v) Keep the oxygen sensor safe whilst the High Temperature disinfection cycle is taking place.
- (vi) To reinstall the oxygen sensor, use the sensor removal tool and install the oxygen sensor by turning it clockwise until it is finger-tight. **NOTE:** Do not use excessive force or any metal tool.
- (vii) Reconnect the sensor by plugging the white connector body back in.
- (viii) Make sure that the sensor wires are inside the metal sensor tube to protect them from damage, then press the rear access cover snugly back in place.
- (ix) After replacing the sensor, humidify the incubator and allow it to stabilise overnight.

5.2.2.2. CO₂ AutoZero

- (i) The CO₂ AutoZero system automatically re-references the CO₂ sensor to atmospheric CO₂. To perform, press the “**USER**” function key and select the “**PEROGRAMMABLE CO₂ AUTOZERO**” and then press “**START**”.
- (ii) The incubator will display a countdown as the AutoZero is running.

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- (iii) When the countdown is complete, the incubator is ready to use.

NOTE: This incubator is fitted with a thermal protection response and it will not run the decontamination routine until this failure has been repaired. See section 5.5 for more details.

5.3. Spillage inside the incubator

If any spillages occur inside the incubator, immediately clean with 70% IMS and refer to SOP038 for further details. If in any doubt, consult the laboratory manager or responsible person.

Before operating the incubator again clean and decontaminate according the procedure described in section 5.2.2

5.4. Equipment malfunction

- (i) If the temperature and/or CO₂ levels deviate more than the programmed setpoints, the display flashes, the audible alarm sounds and a message appears on the screen. You can acknowledge the alarm by pressing any key.

NOTE: When the incubator is switched on, or after the temperature and CO₂ levels have been reprogrammed, the alarm system is inactive until the setpoint values are achieved, after which the alarm system is armed. As setpoint is achieved, the CO₂ and temperature alarms are individually alarmed.

- (ii) After setpoints have been achieved for the first time, when the inner door is opened, the alarm is disabled; on closing the door, if selected, a **programmable alarm delay** starts:
- a. If chamber conditions recover within the programmed alarm delay time, the alarm system is immediately re-armed. After the delay, the alarm system is armed and if the temperature and CO₂ are outside the alarm high and low settings, the alarm will be activated.
 - b. If an alarm occurs and the chamber subsequently recovers, the alarm stops and the system is re-armed. Details of alarm events are stored in the datalogger.
- (iii) If the alarm continues/repeats or any part of the incubator fails or malfunctionsGalx, seek advice from the laboratory manager or responsible person. With permission of the laboratory manager, the user should consult the operating instructions to access fault finding and troubleshooting procedures.
- (iv) If the equipment fails to work or malfunctions and cannot be rectified according to troubleshooting procedures detailed in the Operator and Users Manuals the Laboratory Manager must be informed and a "Do Not Use" notice should be posted on the equipment. Contact the manufacturer for advice and coordinate with the Lab Manager for external maintenance and servicing.

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- (v) External maintenance and servicing of the equipment can only be performed after it has been suitably disinfected (refer to SOP003 for further details) and a 'Decontamination Certificate' has been issued (a pro-forma is available on the CBE LEARN page) and the safety checklist completed (refer to Operators Manual).

5.5. Special notes on Health and Safety

- (i) Observe the relevant biological risk and COSHH assessments associated with the work being carried out and ensure that the correct PPE is worn for the work.
- (ii) Do not use radioactive, toxic or corrosive substances (e.g. chlorine-based substances such as sodium hypochlorite) inside the incubator
- (iii) Do not use substances releasing explosive vapours, except 70% IMS (used for surface disinfection)
- (iv) Wear protective disposable gloves, available in all change rooms, before putting your hands into the incubator.

6. DOCUMENTATION

6.1 QS-form-005 Incubator Calibration Test Record

6.2 QS-form-009 Decontamination of equipment certificate

6.3 Weekly housekeeping record

6.4 Equipment maintenance record

All maintenance and problems should be recorded on the Equipment maintenance and Housekeeping logs.

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SOP Version History

Version Reviewed	Date Revised/ Reviewed	Revision Summary	New Version Number
001	05/10/12 By K.Sikand	Annual review, SOP re-organised to fit the lean SOP framework.	002
002	26/11/15 J.Bowdrey	Removed- “Shall complete the inventory each time they use the incubators” from Section 3. Also changed “Shall record any adverse events and alarms that indicate non-conformance or malfunction on the lab Equipment Maintenance log and notify the Laboratory Manager/Responsible Person.” Removed “NOTE: It is recommended that an inventory of the incubator contents is posted on the front of the incubator, especially when used to quarantine material (see section 8).” From section 5.1 Altered 5.2.2 xx11) to -Record maintenance procedures on the Maintenance of Equipment log and also the house keeping log Removed : QS-form-002 Incubator Preventative Maintenance Record QS-form-007 Incubator Inventory Record QS-form-019 Incubator Cleaning record sheet Added “All maintenance and problems should be recorded on the Equipment maintenance and Housekeeping logs” to Section 6	003
003	21/06/18 Jon Harriman	Reviewed SOP. Slight grammatical changes and updates to latest lab procedures. Changed location to H23. Removed temperature calibration – no longer performed.	004
004	23/06/20	Updated the decontamination section and how to dispose of copper sulphate solution.	005

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