Document Number: SuRF-IMM-007.01

Title: Antigen retrieval using a Digital Decloaking Chamber.

Version 1.0

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Effective from:	01/03/2016
Reviewed annually	

SOP History		
Number:	**Date:**	**Reason for Change:**
01	01/03/2016	Original

1.0 Purpose:

The purpose of this Standard Operating Procedure (SOP) is to describe the current procedure for antigen retrieval using a digital decloaking chamber. Most formalin-fixed tissue requires an antigen retrieval step before immunohistochemical staining can proceed. This is due to the formation of methylene bridges during fixation, which cross-link proteins and therefore mask antigenic sites.

2.0 Scope:

This SOP applies to all staff, including students, visitors and any other supervised / trained individuals involved in this procedure within SuRF Histology based in the Queen's Medical Research Institute (QMRI), Edinburgh, and in accordance with GLP Principles and Regulations.

3.0 Responsibilities:

This document is a guide only – on site training is essential before use

3.1 All staff are responsible for ensuring that methods are followed in accordance with this SOP after suitable training, and where relevant, update their SOP Training Record (Standard document QA008) accordingly.

3.2 All staff involved in this procedure must be familiar with the location of any manufacturer's manuals, instructions or guidance pertaining to equipment or methodology, and are strongly advised to read and understand this material before performing this procedure. This SOP describes the basics of safe and correct use, as well as maintenance of this digital decloaking chamber.

3.3 All staff must have read any corresponding relevant risk assessment / COSHH documents before performing this procedure.

3.4 The responsible person for the digital decloaking chamber within SuRF Histology will be detailed in the responsibilities list.

4.0 Equipment:

4.1 Digital Decloaking Chamber

The Decloaking Chamber is a programmable bench top pressure cooker intended for laboratory use. It is programmed to allow the precise pressurized heating necessary for antigen retrieval and also has the capability to perform at a variety of temperatures ranging from 37°C to 125°C

5.0 Procedure:

Follow operating instructions as per manufacturer's manual.

BRIEFLY

- Plug the Decloaking Chamber into a 110-120 volt power source with surge protection.
- Place the gasket in the lip of the Decloaking Chamber lid.
- Place Decloaking Chamber pan into the Decloaking Chamber shell (body).
- Place the heat shield in the center of the pan.
- Pour 500 ml of deionized (DI) water into the pan.
- Ensure that the heat shield is in the center of the pan after the addition of DI water.
- Place slides into Tissue TekTM racks.
- Place Tissue TekTM racks into Tissue TekTM containers containing 200 ml of desired retrieval buffer (Example: Diva) on top of the heat shield off center to avoid heat concentration. Containers must not be covered.
- Do not use glass containers.
- Place a Steam Monitor Strip across the edge of the Tissue TekTM containers.
- Align the shell and pan handles.
- Match the lid etching "OPEN" with the white dot on the pan handle.
- Hold both the shell and pan handle with your left hand while turning the lid clockwise with the right hand until the lid etching that reads "CLOSED" is aligned with the white dot of the pan handle on the left side.
- The metal tabs on the lid should be tightly seated against the pan's lip.
- Turn the instrument on by flipping the red toggle to the right of the control panel to the "ON" position.
- Press "DISPLAY SET" once, until the SP1 LED illuminates.
- The user can scroll through the instrument settings by pushing the "DISPLAY SET" button multiple times.
- Recommended factory settings for IHC are SP1 of 125° C for 30 seconds, SP2 of 90° C for 10 seconds, SP Safety Limit of 10° C, unless otherwise specified by the data sheet.

- Push “Start/Stop” button to initiate programmed run.
- When the alarm sounds (approximately 10-15 minutes) push “Start/Stop" button. The SP2 will illuminate. Record the time, temperature and pressure.
- When the alarm sounds a second time (approximately 20 minutes) the temperature has cooled to your programmed SP2 temperature.
- Visually confirm that the pressure has dropped to 0 (zero) psi.
- Toggle the weight (petcock) to release any residual pressure. Turn the unit off.
- Open the lid with the steam directed away from yourself.
- Confirm that the Steam Monitor Strip has changed to a dark brown to black colour (Figure 6b).
- Carefully remove the Tissue TekTM containers from the instrument.
- Place the containers on the counter top to cool down for at least 10 minutes.
- Decant half of hot retrieval solution and add DI water.
- Do five changes of DI water.
- Proceed with immunohistochemistry (IHC) staining.

6.0 Related documents / references:

- RA1: SuRF-RA1-IMM-007: Antigen retrieval using a Digital Decloaking Chamber

- COSHH: SuRF-COSHH-IMM-007: Antigen retrieval using a Digital Decloaking Chamber

- Manufacturer’s manuals / instructions

- The University of Edinburgh Health & Safety Policy / Codes of Practice (available on University’s Health and Safety Department website)

- College of Medicine and Veterinary Medicine Health and Safety Manual (available on University’s Health and Safety Department website)

7.0 Approval and sign off:

Author:		
Name:	Lyndsey Boswell	
Position:	Principal Investigator	
Signature:		Date:
Management Approval by:		
Name:	Mike Millar	
Position:	Facility Manager	
Signature:		Date:
QA Release by:		
Name:	Robin Sellar	
Position:	QA Manager	
Signature:		Date:

8.0 Verification by users:

Sign below to indicate you have read and understood the activity outlined above and the risk control measures that you must implement, use and / or wear. Ensure you have received sufficient information, instruction and training to enable you to conduct this activity with the minimum of risk to yourself and others.

Signature:	**Date:**

9.0 Review of assessment:

A suitably trained person should review this assessment at regular intervals and immediately if there is reason to suspect that it is no longer valid (for example after any accidents or incidents) or if there is a significant change in the work to which it relates. If so, a new assessment form must be completed and any original signatories covered by the modified assessment should sign again.

Signature (authorised):	Date: