IMMUNOHISTOCHEMISTRY PROCEDURES USING DAKO PT LINK AND DAKO AUTOSTAINER 48S LINK PLATFORM

PURPOSE AND SCOPE

The purpose of this protocol is to define the procedure for using the automated platform, Dako Autostainer 48S Link for immunohistochemistry, including HIER with the Dako PT Link.

2. RESPONSIBILITIES

2.1 SECTION HEAD BIOMEDICAL SCIENTIST

The BMS in charge of the Immunohistochemistry (IHC) area is responsible for ensuring that the IHC service is maintained to the appropriate standard.

2.2 BIOMEDICAL SCIENTISTS

May carry out the procedure once trained to do so. They are responsible for reporting any faults or staining issues to the section head or advanced biomedical scientist.

2.3 TRAINEE BIOMEDICAL SCIENTISTS AND BIOMEDICAL SUPPORT WORKERS

Trainee staff and BSWs may be called upon to work in the section under the supervision of qualified and trained staff.

3. REFERENCES

EXHIST0020 - PT Link User Guide
EXHIST0021 - PT Link Quick Start Guide
EXHIST0024 – Autostainer Link 48 Quick Reference Guide
EXHIST0022 – Autostainer Link 48 User Guide

4. DEFINITIONS

IHC	Immunohistochemistry
HIER	Heat Induced Epitope Retrieval

5. DOCUMENTATION

5.1 RISK ASSESSMENTS

CPRA 002	Specimen Identification
CPRA 073	Handling Chemicals
CPRA 075	Preparing Solutions
CPRA 081	Preparing Slides for IHC

5.2 COSHH ASSESSMENTS

COSHH 015	Alcohol (IDA99%)
COSHH 385	Xylene
COSHH 240	Pertex
COSHH 266	Proteinase K

5.3 ASSOCIATED FORMS

LF 130 007a	New antibody titration form
LF 130 007b	Existing antibody titration form
LF 130 009	Antibody acceptance record

6 ACTIONS AND METHODS

6.1 PRINCIPLE OF THE METHOD

The Dako staining machines will dewax the slides, perform antigen retrieval where required and then carry out an immunohistochemical staining technique. In order to ensure optimal staining results, the appropriate antigen retrieval and antibody dilution need to be worked out for each new antibody. In some cases, variation between antibody batches/lot numbers may mean that existing antibodies sometimes need re-titrating.

6.2 SPECIMEN REQUIREMENTS

- Correctly labelled 4µ paraffin sections mounted on 'Superfrost Plus' slides.
- Appropriate positive and negative control material.

6.3 DATA ACQUISITION

- Antibody data sheets.
- Antibody titration forms (LF 130 007a & b)

6.4 REAGENTS, CONTROLS AND EQUIPMENT REQUIRED

Relevant control slides for each antibody to be stained
User fillable reagent pots
The relevant antibody
Antibody diluents
Pipettes/ Pipette tips

6.5 CALIBRATION

All calibration is carried out by service engineers.

6.6 STEPWISE DESCRIPTION OF THE PROCEDURE

6.6.1 Cutting sections for Immunohistochemistry.

- Sections should be cut at 4 µm onto superfrost slides with appropriate control section on the same slide, following the SOP LP130 007 Cutting Sections for Immunohistochemistry.
- Pre heat the slides in the 60°C oven for a minimum of 30 mins.
- Once the slides are ready generate slide labels as instructed in the Dako Autostainer user guide EXHIST0022.

6.6.1 Preparing slide labels

- Information to be added to slide labels should include:
    - The lab number and surname of the Patient.
    - Select the pathologist that has requested the slides
    - Select the antibody that has been requested

- Once all the antibodies for that request have been selected, press print labels and then case complete.

- The name of the antibody will automatically appear on the slide due to the way the labels are generated by the Dako system.

- The labels must be stuck centrally in the label area of the slide and kept as straight as possible.

- Rack the slides in the slide racks for the PT Link, separating the slides into the high and low pH retrieval solution.
- Slides that require Proteinase K pre-treatment must be dewaxed and taken to water prior to staining, do not put these slides into the PT Link. Once dewaxed the slides can put directly on the Autostainer. Make sure that the Proteinase K container is added to the reagents to be loaded onto the Autostainer.

6.6.2 Antigen Retrieval using the PT LInk

- Refer to:
    - EXHIST0020 and EXHIST0021 for how to use the PT Link

Chemical-protective gloves should be worn when handling parts immersed in any reagent used in PT Link.

- Fill tanks with desired Dako Target Retrieval Solutions (Codes S1699/S2367).
- Place slide racks with slides into tanks.
- Close and lock lid with external latch.
- Press **RUN** button for each tank to start run.
  a. If Preheat is enabled, CYCLE will show **PREHEAT 65°C**.
  b. Press **RUN** again to start the run, CYCLE will show **WARMUP** and the lid lock will engage.
- Unit will warm up to set temperature and then start countdown clock for retrieval cycle.
- When retrieval cycle is finished, CYCLE will show **COOL**.
- When COOL cycle is finished, CYCLE will show **DONE** and lid will unlock.
- Press **DONE** on the Dako Link software to acknowledge that you have removed the slides.
- Take one slide rack at a time out of PT Link and rinse all slides with warm (65°C) Dako Wash Buffer (Code S3006).
- Rinse slides with room temperature Dako Wash Buffer.
- Place the rack on a Dako Autostainer Link instrument.

6.6.3 Performing Immunohistochemistry with Dako Autostainer Link

- Prepare reagents required and load them onto the Dako Autostainer Link.
- Remove the lids and place them on the place holder under the instrument so that they match the position of the reagents. This will make sure that the lids are not mixed up when the run is complete and avoids contaminating the reagents.
- Make sure that the bulk reagents containers have been filled up with wash buffer and water, and that the hazardous waste container has sufficient capacity to hold the hazardous waste during the run.
- Prime the bulk solutions with the first run of the day following the on-screen instructions.
- Press **START** to start the run. The machine will scan all the slides and reagents and inform you if all the reagents that are required for the run are there. If not, it will tell you which reagents are required and then ask you to press **OK** and rescan the reagents.
- Once all the reagents are present, press start to start the run. The machine will ask you if you have enough bulk fluids and ask you to press **YES**

- At the end of the run press **DONE** and remove the slides and reagents.
- Remove the slides from the racks and transfer them to the racks for the coverslipper.
- Place slides in water to wash for 5 minutes, then dehydrate with 70% Alcohol, two changes of Absolute Alcohol and two changes of Xylene.
- Coverslip the slides on the Leica coverslipper CV5030.

6.7 QUALITY CONTROL / ASSESSMENT

The requesting Pathologist / Consultant Pathologist along with the Section Lead must evaluate the slides produced before a new antibody is added to the repertoire.

6.8 LIMITATIONS OF METHOD

The effects of antigen retrieval may vary with the length of time of fixation or the type of fixative used. These things are not always known, particularly with referred material.

All staff performing these tasks should be fully trained for the tasks set out above and have competency records to prove this, before they can perform the tasks unassisted.

6.9 REPORTING OF RESULTS AND INTERPRETATION

Reporting of results can only be carried out by the clinical pathologists. Biomedical Scientists and Biomedical Support workers cannot issue reports on Immunohistochemistry results. The Lead Biomedical Scientist must check the quality of the immunostaining on the control tissue before sending the slides to the requesting pathologist.

6.10 PROCEDURE NOTES AND OTHER PERTINENT INFORMATION

It is essential that control material used for antibody titration is selected carefully. Ideally, known positive AND known negative tissues should be used. Appropriate control material is usually suggested on the antibody data sheet and is crucial to identify genuine positive staining. If an antibody titre is too concentrated, non-specific staining may occur in known negative tissues while still staining appropriately on the positive control material. If possible a range of positive tissues should be used to include high, moderate and low expressors in order to correctly determine the optimum titration.

7. TRACEABILITY AND UNCERTAINTY

7.1 TRACEABILITY

All antibodies have product inserts uploaded to Q-Pulse which provide information on dilutions, pre-treatments and control material and which can be used as reference material if required. Guidance on how to use the machines is traceable back to the manufacturer user manuals.

7.2 UNCERTAINTY

Please refer to QP 000 036 Measurement of Uncertainty and Criticality in Cellular Pathology